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APPEAL BRIEF

Attorney Docket No. 30775-701.403

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the Application of:	
Applicant: Jonathan W. Nyce	Confirmation No.: 5176
Serial No.: 10/072,010	Group Art Unit: 1617
Filed: October 25, 2001	Examiner: San Ming R. Hui
Title: **Compositions For Treatment Of Asthma Or Bronchoconstriction**	Customer No. 021971

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APPELLANT'S BRIEF PURSUANT TO 37 C.F.R. § 41.37

Appellant submits this brief in accordance with the provisions of 37 C.F.R. § 41.37 in response to the Final Rejection mailed April 12, 2006. Appellant's Notice of Appeal and Pre-Appeal Conference Brief was filed May 31, 2006. A Notice of Panel Decision from Pre-Appeal Brief Review was mailed July 27, 2006. The Panel Decision was to proceed to the Board of Patent Appeals and Interferences.

The fee for filing a Brief in Support of an Appeal under 37 C.F.R. §41.20(b)(2) is electronically submitted herewith. A Petition for Extension of Time is requested for a reply within the first month and the fee set forth under 37 C.F.R. §1.17(a)(1) is electronically submitted herewith. This Appeal Brief is therefore timely filed.

I. REAL PARTY IN INTEREST

The real party in interest is East Carolina University (Assignee) by virtue of an assignment executed by the inventor (Appellant) to East Carolina University (recorded by the Assignment Branch of the U.S. Patent and Trademark Office on April 13, 1995 at Reel/Frame 007450/0084).

II. RELATED APPEALS AND INTERFERENCES

Appellant is filing an Appeal Brief in accordance with the provisions of 37 C.F.R. § 41.37 in co-pending Application No. 10/410,955, "Methods For The Treatment Of Asthma Or Brochoconstriction" As of the filing of this Appeal Brief, no decisions have been rendered by a court or by the Board on that matter.

III. STATUS OF CLAIMS

The application under appeal currently includes claims 160-162, 165, and 187-190. Claims 160-162 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Prendergast U.S. Patent 4,956,355 (Prendergast), in view of Lieberman et al., "Pharmaceutical Dosage Forms", page 110, (Lieberman) and Gennaro, Alfonso R., "Remington: The Science and Practice of Pharmacy", 17th Ed., 1985, page 1505 (Remington).

Claims 187-189 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Prendergast, Lieberman and Remington as applied to claims 160-162, 165 above, and further in view of Kelly et al., "Chapter 24/Asthma", 2nd Ed., 1992, pages 408-449 (Kelly).

Claims 160-162, 165 and 187-190 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Nyce U.S. Patent 5,527,789 (Nyce) in view of Lieberman., Remington, and Kelly. Claims 160-162, 165, and 187-190 are appealed.

IV. STATUS OF AMENDMENTS

Appellant has submitted no amendments after the final rejection. All amendments prior to the close of prosecution on the merits have been entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 160 recites a pharmaceutical composition, comprising a carrier and an amount of active agent effective for treatment of bronchoconstriction, lung inflammation, lung allegy, or asthma. The agent is selected from dehydroepiandrosterone, or pharmaceutically or veterinarily acceptable salts thereof. The dehydroepiandrosterone is based on the chemical formula shown in formula (I). The composition comprises <u>particles of about 1.0 μm to about 5 μm in size</u>.

The compounds based on the dehydroepiandrosterone of formula (I) are described in the specification at least on page 7, line 20 to page 9, line 8. The carrier is described in the specification at least on page 9, line 19, page 10, line 7 to 11, page 12, line 25 to page 13, line 7, and page 13 lines 21-26. Amounts of active agent effective for treatment of bronchoconstriction, lung inflammation, lung allegy, or asthma are described in the specification at least on page 7, lines 14-20, page 8, lines 19-22, and page 10, line 16 page 11, line 5. The composition comprising particles of about 1.0 µm to about 5 µm in size is described in the specification at least on page 14 lines 21-22.

Dependent claims 161, 162, and 165 depend on independent claim 160. Dependent claim 165 additionally recites an amount of ubiquinone (CoQn, wherein n=1 to 12) effective to reduce adenosine depletion. The ubiquinone is described in the specification at least on page 8 lines 16-22, and on page 9 line 24 to page 10 line 6. Reduction of adenosine depletion is described in the specification at least on page 11, lines 6-20, and in Examples 1 and 2, page 16, line 8 to page 17, line 8.

Independent claim 187 recites a pharmaceutical composition comprising a carrier and an amount of an active agent effective for treatment of bronchoconstriction, lung inflammation, lung allergy, or asthma selected from dehydroepiandrosterone, or pharmaceutically or veterinarily acceptable salts thereof. The dehydroepiandrosterone is based on chemical formula (I). The pharmaceutical composition comprises particles about 15 μm to about *500* μm in size. The compounds based on the dehydroepiandrosterone of formula (I) are described in the specification at least on page 7, line 20 to page 9, line 8. The carrier is described in the specification at least on page 9, line 19, page 10, line 7 to 11, page 12, line 25 to page 13, line 7, and page 13 lines 21-26. Amounts of active agent effective for treatment of bronchoconstriction, lung inflammation, lung allegy, or asthma are described in the specification at least on page 7, lines 14-20, page 8, lines 19-22, and page 10, line 16 page 11, line 5. The pharmaceutical composition comprises particles about 15 μm to about *500* μm in size is described in the specification at least on page 14, lines 25 -29.

Dependent claims 188-190 are dependant on independent claim 187. Claim 190 additionally recites an amount of ubiquinone (CoQn, wherein n=1 to 12) effective to reduce adenosine depletion in an animal tissue. The ubiquinone is described in the specification at least on page 8 lines 16-22,

and on page 9 line 24 to page 10 line 6. Reduction of adenosine depletion in animal tissue is described in the specification at least on page 11, lines 6-20, and in Examples 1 and 2, page 16, line 8 to page 17, line 8.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellant respectfully requests the Board of Patent Appeals and Interferences to review the following grounds of rejection on appeal:

1. Whether claims 160-162 are patentable under 35 U.S.C. 103(a) Prendergast in view of Lieberman and Remington.
2. Whether claims 187-189 are patentable under 35 U.S.C. 103(a) over Prendergast, Lieberman, and Remington, further in view of Kelly.
3. Whether claims 160-162, 165 and 187-190 are patentable under 35 U.S.C. 103(a) over Nyce in view of Lieberman, Remington, and Kelly.

VII. ARGUMENT

Appellant respectfully submits that claims 160-162, 165, and 198-190 are in proper form and are patentable over the prior art of record.

1. The Examiner erred in rejecting claims 160-162 under 35 U.S.C. 103(a) as being unpatentable over Prendergast in view of Lieberman and Remington

The Examiner states, in the final office action of Mar. 2, 2006, that Prendergast discloses "that particular dehydroepiandrosterones (DHEA) herein are useful in a pharmaceutical composition or a pharmaceutical formulation of enteral, parenteral, injectable, topical, inhalations, or nasal inhalation administration", (page 2) but that Prendergast "does not expressly disclose the particular ranges of particle size herein, about 1.0-5μm in size." (page 3) The Examiner states that Lieberman "teaches that a skilled artisan in pharmaceutical science would clearly knowledge that the granulation, determination of size, or size reduction of a solid pharmaceutical formulation, e.g., in nasal inhalation formulation, have several benefits, for example as taught" in Lieberman's text book (page 3). The Examiner states that Remington "teaches that the optimum particle size for preparation into the pulmonary cavity is on the order of ½ to 7μm." (page 3). The Examiner further states that "it would have been obvious to a person of ordinary skill in the art at the time the

invention was made to determine and granulate the dehydroepiandrosterone sulfate particles in range of size herein for nasal inhalation." (page 4). The Examiner further states that "One having ordinary skill in the art at the time the invention was made would have been motivated to determine and granulate the dehydroepiandrosterone sulfate particles in range of size herein for nasal inhalation, since particular dehydroepiandrosterone sulfate (DHEA-S) are known to be pharmaceutical compositions for inhalations or nasal inhalation administration based on Pendergast". (page 4).

The Examiner fails to make a prima facie case of obviousness because there is no suggestion or motivation in the prior art to combine the Prendergast, Lieberman, and Remington references. A proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to one of ordinary skill in the art that he should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, one of ordinary skill would have a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

There is no suggestion or motivation to combine Prendergast with Remington because Prendergast is directed mainly to <u>systemic</u> treatment and only mentions inhalation as part of a laundry list of treatment options. The prior art as a whole must make the invention obvious. *Hartness v. Simplimatic Eng'g,* 819 F.2d 1100, 1108, 2 USPQ2d 1826, 1832 (Fed.Cir.1987). The Federal Circuit has held that it is improper to "pick and choose among the individual elements of assorted prior art references to recreate the claimed invention," but rather, one looks for "some teaching or suggestion in the references to support their use in the particular claimed combination." *Smithkline Diagnostics v. Helena Laboratories*, 859 F.2d 878, 887, 8 USPQ2d 1468, 1475 (Fed.Cir.1988). Prendergast, which is titled "Agents for the Arrest and Therapy of Retroviral Infections" is directed toward the use of DHEA-type compounds for the treatment of retroviral infections such as HIV. In the treatment of retroviral infections, it is typically important that the compound be administered such that it is present <u>systemically</u> throughout the whole body. In Prendergast, the examples only describe an oral formulation of DHEA-S via a capsule. The only reference that Prendergast makes to inhalable formulations is as one of a laundry list of methods of using the drug. It is possible for a prior art reference to include a wide variety of species, but not to

disclose a particular subject matter, as here claimed. See *In re Ruschig,* 379 F2d 990, 154 USPQ 118 (CCPA 1967). The legal question is: Whether or not it can be fairly and reasonably said that one of ordinary skill in this art through a reading of the entire reference has constructive possession of the claimed composition itself, as opposed to the possession of mere language which somehow embraces the name of what may be claimed. See *In re Lavisi et al.*, 144 USPQ 646 at page 650. Genarro, as a whole, teaches numerous methods of drug delivery and numerous types of pharmaceuticals. Table 1 shows a partial list of the modes of drug delivery and of pharmaceutical and medicinal agents described in Remington (see Evidence Appendix). The Table gives a sense of the total teaching of Remington, which provides no indication that the specific drug DHEA, only shown in Koo to be orally delivered, should be delivered by any of the other modes described. Therefore, considering the teaching of Prendergast as a whole, there is no motivation or suggestion to combine Prendergast with Remington, where Remington provides only a teaching of many methods and many drugs.

It is improper for the Examiner to use the present application as the motivation to combine the references. Both the suggestion and the reasonable expectation of success "must be founded in the prior art, not in the applicant's disclosure." *Vaeck*, 947 F.2d at 493. The present invention relates specifically to pharmaceutical formulations using DHEA-S for treating bronchoconstriction, lung inflammation, lung allergy, or asthma. The time of the invention was in 1995, over 11 years ago, and at that time, the use of DHEA-S for treating these types of diseases, for example, through an inhalation route was not known. The Examiner, in reading Appellant's specification can see the value of the use of these particular compounds for these particular types of diseases via an inhalation method, but it is improper for the Examiner to use the knowledge from this specification in order to establish a motivation. To do so uses impermissible hindsight. Thus no motivation or suggestion outside the present specification to combine Prendergast and Lieberman or Remington has been established.

Lieberman does not suggest a combining the references because Lieberman teaches that the particle size used varies greatly with the specific compound that is being made into particles. The Examiner uses Lieberman to argue that the particular range claimed is obvious, but reviewing

sections of Lieberman not cited by the Examiner leads to the opposite conclusion. On page 32 of Lieberman, not quoted by the Examiner, there is a description of how one must pay attention to the composition (physicochemical structure), size (and size distribution), and shape of particles. The text states that "the molecular composition and arrangement distinguishes it from all other materials, and dictates its behavior as part of the powder" (Lieberman, page 32). Lieberman says "each powder has a "critical size" where cohesive forces begin to affect the flow properties." (Lieberman, page 35). Lieberman presents a table that demonstrates how different molecular compositions give rise to very different properties in the same size powders (see Lieberman pages 36-37). Thus Lieberman does not teach that one can routinely determine the effective particle size for a new compound by looking at the particle size of other compounds with different chemical structures. Lieberman demonstrates how unpredictable and sophisticated the granulation of pharmaceuticals is. Thus, the Examiner erred in combining Prendergast with Lieberman and Remington.

Lieberman also should not be combined with Prendergast and Remington because in some sections, it teaches away from particle sizes below 10μm. It is improper to combine references where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 744, 218 USPQ 769, 780 (Fed. Cir. 1983). Lieberman states, in a section not cited by the Examiner, that the particle size and particle size distribution have "considerable impact on the flow properties of powders," how large dry particles flow better than smaller particles because they have greater mass, how smaller particles may create mixing problems "because surface areas are very great," and how as "the particle size approaches 10 μm and below, weak polarizing electrical forces called van der Waals and electrostatic forces"..."inhibit powder flow through particle agglomeration". (Lieberman, pages 32-33). Lieberman also says that "cohesive forces are strong in powders composed of particles 10μm or less in size (Lieberman, page 35). Thus, sections of Lieberman caution about the use of particles below 10 μm because of adverse powder properties. As described above, the prior art must be assessed as a whole. Therefore, the Examiner erred in combining Lieberman, Prendergast and Remington because parts of Lieberman teach away from the combination.

Appellant has presented evidence of unexpected results sufficient to rebut a prima facie case of obviousness, and the Examiner has failed to adequately address those results. Where a prima

facie case of obviousness has been established, the patent applicant can rebut the prima facie case by a showing of unexpected results, i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. *In re Soni*, 54 F.3d 746, 750, 34 U.S.P.Q.2d 1684, 1687 (Fed. Cir. 1995). The unexpected results must be established by factual evidence. Mere argument or conclusory statements in the specification does not suffice. *In re De Blauwe*, 736 F.2d 699, 705, 222 USPQ 191, 196 (Fed.Cir.1984). Here, a declaration was submitted by an expert in the field, Dr. Cynthia Robinson, including extensive experimental work, including experiments with humans as well as with dogs showing unexpected results for compounds of the present invention in the particle size range of about 1.0-5μm. The compounds, when used in this particle size range, unexpectedly showed the efficacy of inhaled DHEA-S in treating asthma while producing minimal adverse side effects. The unexpected results included seeing only a modest increase in circulating DHEA-S at dosages that were effective to treat asthma. These results are significant because in treating asthma with DHEA-S it can be very desirable to effectively distribute the compound locally within the lung, but to minimize the amount of the compound that is introduced systemically due to the known, undesirable side effects of the sterioidal compounds. Dr. Robinson described the results as unexpected because "it would be expected that the greater access to the systematic circulation in the lungs would cause systemic absorption and result in systemic side-effects such as modified levels of sex hormones and/or adverse effects on sex organs." Thus, evidence has been provided that establishes unexpected results sufficient to rebut a prima facie case of obviousness.

The Examiner has failed to adequately address the unexpected results that were presented. Where an applicant demonstrates substantially improved results and states that the results were unexpected, this should suffice to establish unexpected results in the absence of evidence to the contrary. *Soni*, 54 F.3d 746 at 751. Here, the Examiner's response to the unexpected results was simply to repeat his prior argument and to assert that the results were insufficient because "the declaration provides no <u>side-by-side</u> comparison with the closest prior art". (see Advisory Action, May 20, 2005. The Examiner provides no case law or statutory basis for requiring such a "side-by-side" comparison. This is one of several instances where the Examiner imposes requirements that

are not supported with any case law or statutory basis. Appellant is aware that "when unexpected results are used as evidence of nonobviousness, the results must be shown to be unexpected compared with the closest prior art," *In Re Baxter Travenol Labs.*, 952 F.2d 388, 392 (Fed.Cir.1991), but this requirement does not mandate side-by-side testing. Here, the unexpectedness in the results relates to the effectiveness of the compound in treating asthma, while at the same time having minimal side effects, which has been shown. In the Final Office Action, the Examiner responds to the presented unexpected results by stating: "the alleged unexpected benefit is probably well known". (emphasis added)(see Final Office Action of Mar. 2, 2006, page 10). Again, the Examiner asserts a standard that does not appear to be legally defensible by dismissing the results as "probably" well known. Thus, the Examiner erred by failing to address the unexpected results presented during prosecution sufficient to rebut a prima facie case of obviousness.

The Examiner's contention that the selection of particle size is merely an optimization is wrong. The Examiner contends that it is "within the skill in the art to select optimal parameters, such as the amounts of ingredients, in order to achieve a beneficial effect." (see Final Office Action of Mar. 2, 2006, page 5). The Examiner cites *In re Boesch,* 617 F.2d 272, 205 USPQ 215 (CCPA 1980) to support this contention. However, *Boesch* states the rule that "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." (emphasis added) *Boesch,* 617 F.2d 272 at 276. It can hardly be said that making a formulation for treatment asthma is a "known process". The area of formulation of inhalable drugs is a sophisticated and unpredictable technology. This can be appreciated by viewing the considerations described in Dr. Robinson's declaration that determining a particle size for a certain set of biological activities is not an optimization activity that can be determined by consulting a textbook. It can also be seen in the passages of Lieberman discussed above describing the complex considerations in creating a powdered pharmaceutical. The literature at the time of the invention would not have made it obvious that this compound in the claimed particle size range would provide effective treatment of asthma, yet result in a low level of compound in the bloodstream. Thus, the Examiner erred in finding the claims obvious as a mere optimization of parameters.

The area of formulation of inhalable drugs is a sophisticated and unpredictable technology, favoring a decision of patentability. It is a well established patent law that "a considerable degree of unpredictability" in a field allows for a decision in favor of patentability. See *In re Schechter*, 205 F.2d 185, 191, 98 U.S.P.Q. 144, 150 (CCPA 1953). In *In re Schechter*, the court reversed a rejection of unpatentability to a group of compounds over prior art isomers. The court took biological function into account in deciding in favor of patentability. There are various types of problems that are encountered in formulating an inhalable drug, including choosing a particle size for a drug, the compatibility of the particle size with the delivery device and the suitability of the particle size and the delivery device in the treatment of a respiratory disease. The inventor has discovered a particular set of compounds in a particular range of sizes was particularly effective in the background of an unpredictable technology. Therefore, the specific compositions claimed are not obvious in light of the unpredictability of the field.

It is improper for the Examiner to assert that the use of "conventional techniques" to make these formulations is obvious. In the Final Office Action, the examiner states that it is "the examiner's position that it is obvious to one of ordinary skill in the art that using <u>conventional techniques</u> to make inhalable, respirable or nasal formulation of the known active agents are considered well within the skill of the artisan in pharmaceutical science." (see Final Office Action of Mar. 2, 2006, page 4). The Examiner does not state any legal basis for asserting that the use of conventional techniques to create formulations would render otherwise novel formulations obvious, and it appears difficult to support this position. Therefore, the Examiner was wrong in asserting that the use of <u>conventional techniques</u> to create the formulations in the claims renders them obvious.

The Examiner erred in concluding that the compositions of Prendergast "intrinsically comprise" DHEA-S particles of about 1-5μm in size. In the Final Office Action, the Examiner states "Thus, the dehydroepiandrosterone sulfate compositions of Prendergast for inhalations or nasal inhalation <u>intrinsically comprise</u> dehydroepiandrosterone sulphate particles having about 1-5μm in size." (see Final Office Action of Mar. 2, 2006, page 4). Again, the Examiner asserts no legal basis for asserting applying an "intrinsically comprise" test in an obviousness rejection under § 103. The facts the examiner uses to arrive at this conclusion are no different than those cited above in support

of this obviousness rejection. Thus, the Examiner has added nothing to his obviousness argument with this assertion, and therefore it does not establish the obviousness of the claims.

The Examiner improperly cited art that was published subsequent to the filing of the patent application. In the Final Office Action, the Examiner cites two publications that have publication dates subsequent to filing of the patent application. One, Genarro, Alphonso R., Remington: The Science and Practice of Pharmacy 20th Ed. page 735, according to the Examiner "teaches the optimum size for inhalations is known to be 0.5 to 0.7μm." The other, Ansel et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Ed., pages 454-455 "teaches that the fine particle size for inhalations is known to range 0.5 – 5μm." (see Final Office Action of Mar. 2, 2006, page 7). The Examiner does not refer to them as the basis of the 103 rejection, but he proceeds to include the references in his argument. In certain circumstances, references cited to show a universal fact need not be available as prior art before applicant's filing date. *In re Wilson*, 311 F.2d 266, 135 USPQ 442 (CCPA 1962). Here, however, the Examiner is not establishing a well known truth, but attempting to show that specific particle size ranges were known in the prior art. The Examiner states that he is using these references "to merely point out the various factors that need to be considered when formulating aerosol formulations," but that is not how they are used in his argument; they are used to establish the prior art (see Final Office Action of Mar. 2, 2006, page 7). Thus, these references cited by the Examiner should be removed from consideration with respect to the obviousness of the present invention.

For the reasons cited above, the Examiner has not presented a prima facie case for obviousness. Additionally, the inventor has presented evidence of unexpected results sufficient to rebut prima facie obviousness and the evidence of unexpected results has not been addressed by the Examiner as required. Therefore, the rejection based on obviousness is in error, and the rejection should be reversed.

2. The Examiner erred in rejecting claims 187-189 under 35 U.S.C. 103(a) as being unpatentable over Prendergast, Lieberman and Remington as applied to claims 160-162, 165 above, and further in view of Kelly

In stating this rejection of claims 187-189, the Examiner first improperly asserts a conclusion that "Prendergast, Lieberman, and Remington teach the composition of DHEA-S in the particle size

as about 1-5µm." (see Final Office Action of Mar. 2, 2006, page 5). The Examiner then states that the references do not expressly teach the particle size of DHEA-S as 15-500µm, and that Kelly teaches the devices for delivering therapeutic aerosols generate particles with aerodynamic diameters of 0.5-35µm in diameter. The Examiner states that one of ordinary skill in the art would have been motivated to formulate the particle size of the DHEA-S composition to 15-500µm because it is known that particle size of 0.5-35µm in diameter as useful in delivering drug particle into the lung. (Final Office Action of Mar. 2, 2006, page 5).

The obviousness of compounds at particle sizes of about 1-5µm does not support the obviousness of compounds at about 15-500µm. While it is improper for the Examiner to base his argument on the "conclusion" that "Prendergast, Lieberman, and Remington teach the composition of DHEA-S in the particle size as about 1-5µm" (Final Office Action of Mar. 2, 2006, page 5), this conclusion does not support the Examiner's subsequent conclusion that a compositions of the present invention in particle size range of about 15-500µm must be obvious. A range of 1-5µm is distinct from the range of 15-500µm, so the teaching of one range, if anything, would teach away from the other. Thus the combination of Prendergast, Lieberman, and Remington does not support an obviousness rejection of claims 187-189. One aspect of the Examiner's argument appears to be that since past examples can be found of making pharmaceuticals in a wide range of particle sizes in the past then particles of any new compound with a size within these past ranges is obvious. If this reasoning were to hold, then one could never patent a particular particle size range for a particular composition, because a different compound from the past could always be found at that size. Thus, an argument by the Examiner that particle sizes is known for a past pharmaceuticals is not sufficient to make this particular size range for this particular set of compounds obvious.

There is no suggestion or motivation to combine Kelly with Prendergast, Lieberman, and/or Remington. The portion of Kelly that was cited by the Examiner because "it is known that particle size of 0.5-35µm in diameter as useful in delivering drug particle into the lung." However, the section of Kelly does not say what the Examiner states. The section of Kelly cited by the examiner says "Particles greater than 10 µm deposit in the oropharynx". The oropharynx is at the back of the mouth. Thus the Examiner's contention that Kelly teaches particles in the size range of about 15-

500µm, going to the lung does not fairly represent the reference and is wrong! Thus, the Examiner's argument that Kelly teaches introduction into the lungs does not provide the motivation to combine the references so as to make compounds of the present invention in the size range of about 15-500µm obvious.

In addition, the combination of the Remington, Lieberman and Prendergast is also not suggested for the same reasons as argued for the first § 103 rejection above.

For the reasons cited, the Examiner has not presented a prima facie case for obviousness. Therefore, the rejection based on obviousness is in error, and the rejection should be reversed.

3. The Examiner erred in rejecting claims 160-162, 165 and 187-190 under 35 U.S.C. 103(a) as being unpatentable over Nyce in view of Lieberman, Remington, and Kelly

In making this last § 103 rejection, Examiner again applies Lieberman, Remington, and Kelly, but this time the references are combined with Nyce rather than with Prendergast. This rejection, unlike those above, is directed both at claims relating to particles about 1-5µm in size (claims 160-162, and 165), and claims relating to particles about 15-500µm in size (claims 187-190). In addition, this rejection is the only rejection that covers dependent claims 165 and 190, both of which recite compositions comprising an amount of ubiquinone. The Examiner states that Nyce discloses a pharmaceutical composition comprising the instant DHEA and the instant ubiquinone. The Examiner states that Nyce discloses a nasal spray, oral, rectal, topical, transdermal, nasal, or parenteral including injectable and in solution. He states that Nyce does not expressly disclose particles of the active agent having sizes of either about 1-5µm, or about 15-500µm. The Examiner states that "one having ordinary skill in the art would have been motivated to determine and granulate the dehydroepinadrosterone particles in range of size herein for nasal inhalation, since the nasal formulation or composition comprising two instant active agents is known based on Nyce."

With respect to the obviousness of claims 160-162 and claims 187-189, because Nyce adds no more than Prendergast, all of the arguments above that these claims are not obvious apply here. Nyce provides nothing more than Prendergast to provide a motivation or suggestion to combine Lieberman, Remington or Kelly. As in Prendergast, Nyce gives a laundry list of potential modes of treatment with DHEA, this list of methods does not provide a teaching with respect to any particular

particle size range. The Examiner appears to include Nyce in this rejection for the reason that Nyce includes ubiquinone, which is not relevant to claims 160-162 or claims 187-189. Therefore the same arguments made above with respect to Claims 160-162 and claims 187-189 apply to this §103 rejection, and that these claims do not become obvious by substituting Nyce for Prendergast.

This rejection differs from those above in that it attempts to use the same set of references to reject both claims to claims of about1-5μm in size and claims to particles of about 15-500μm in size. The Examiner's argument is, therefore, not that either of the particular size ranges themselves is obvious, but that it would have been obvious to one of ordinary skill in the art to determine the effective size range. This assertion by the Examiner is insufficient to provide a basis for obviousness. As described above, Lieberman makes it clear that the selection of a specific particle size of a pharmaceutical formulation is not routine, and depends on many factors including the chemical composition of the pharmaceutical itself. Therefore it would not be obvious to formulate the compounds of the present invention in the claimed size ranges.

As described above, the Examiner's contention that the selection of particle size is <u>merely an optimization</u> is in error. *Boesch,* cited by the Examiner states the rule that "discovery of an optimum value of a result effective variable in a <u>known process</u> is ordinarily within the skill of the art." (emphasis added) *Boesch,* 617 F.2d 272 at 276. However, making a formulation for treatment asthma with a new drug is not a "known process", and therefore determining an effective particle size for a new drug is not routine. In addition the area of formulation of inhalable drugs is a sophisticated and unpredictable technology, favoring a decision of patentability. A "considerable degree of unpredictability" in a field allows for a decision in favor of patentability. *Schechter*, 205 F.2d 185, 191. There are various types of problems that are encountered in formulating an inhalable drug, including choosing a particle size for a drug, the compatibility of the particle size with the delivery device and the suitability of the particle size and the delivery device in the treatment of a respiratory disease. The inventors have discovered a particular set of compounds in a particular range of sizes was particularly effective in the background of an unpredictable technology. Therefore, the specific compositions claimed are not obvious in light of the unpredictability of the

field. For the reasons cited, the rejection based on obviousness is in error, and the rejection should be reversed.

The Examiner erred in rejecting dependent claims 165 and 190 under 35 U.S.C. 103(a) as being unpatentable over Nyce in view of Lieberman, Remington, and Kelly

Appellant in this Appeal Brief has argued the claims as a group and has not argued each dependent claim separately. Appellant provides arguments here for dependent claims 165 and 190 here because the Examiner explicitly discusses ubiquinone, which only relates to these two claims (see Final Office Action page 6).

Neither of dependent claims 165 and 190 is obvious under Nyce in view of Lieberman, Remington, and Kelly. Claim 165 is dependent on claim 160, which claims DHEA type compounds comprising particles of about 1-5μm in size. Therefore, since claim 160 is not obvious in view of the cited art for the reasons cited above, claim 165 is not obvious. In addition, claim 165 recites the limitation of an amount of ubiquinone effective to reduce adenosine depletion. Nyce does not address adenosine depletion, and adenosine depletion is not described in Lieberman, Remington, and Kelly. Therefore the use of ubiquinone in amounts effective to reduce adenosine depletion is not obvious in view of the cited references. Claim 190 is dependent on claim 187, which claims DHEA type compounds comprising particles about 15-500μm in size. Therefore, since claim 187 is not obvious in view of the cited art for the reasons cited above, claim 190 is not obvious. In addition, claim 190 recites the limitation of an amount of ubiquinone effective to reduce adenosine depletion in animal tissue. Nyce does not address adenosine depletion generally or adenosine depletion in animal tissue, and neither are these described in Lieberman, Remington, and Kelly. Therefore the use of ubiquinone in amounts effective to reduce adenosine depletion in animal tissues is not obvious in view of the cited references. Thus, dependent claims 165 and 190 are not obvious under Nyce in view of Lieberman, Remington, and Kelly.

For the reasons cited, the Examiner has not presented a prima facie case for obviousness. . Therefore, the rejection based on obviousness is in error, and the rejection should be reversed.

CONCLUSION

For the reasons stated above, claims 160-162, 165, and 187-190 are patentable over the prior art of record, and the rejections to those claims under 35 U.S.C. § 103 are improper. Appellant respectfully requests the Board to reverse the Examiner's rejections with instructions to allow the claims.

The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 23-2415 (Attorney Docket No. 30775-701.403).

Respectfully submitted,

Date: December 22, 2006 By: Albert P. Halluin

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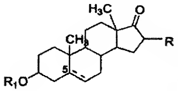
VIII. CLAIMS APPENDIX

1-159. (Cancelled)

160. (Previously Presented) A pharmaceutical composition, comprising a carrier and an amount of an active agent effective for treatment of bronchoconstriction, lung inflammation, lung allergy, or asthma selected from dehydroepiandrosterone, or pharmaceutically or veterinarily acceptable salts thereof, the dehydroepiandrosterone having the chemical formula



(I) ;

wherein the broken line represents a single or double bond; R is hydrogen or halogen; the H at position 5 is present in the alpha or beta configuration or the compound of chemical formula I comprises a racemic mixture of both configurations; and R_1 is SO_2OM, wherein M is H,

wherein the pharmaceutical composition comprises particles of about 1.0 μm to about 5 μm in size.

161. (Previously Presented) The pharmaceutical composition of claim 160, wherein said active agent is dehydroepiandrosteronesulfate.

162. (Previously Presented) The pharmaceutical composition of claim 160, which is an inhalable or nasal formulation.

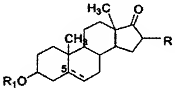
163. (Cancelled)

164. (Cancelled)

165. (Previously Presented) The pharmaceutical composition of claim 160, further comprising an amount of ubiquinone (CoQn, wherein n=1 to 12) effective to reduce adenosine depletion.

166 - 186. (Cancelled)

187. (Previously Presented) A pharmaceutical composition, comprising a carrier and an amount of an active agent effective for treatment of bronchoconstriction, lung inflammation, lung allergy, or asthma selected from dehydroepiandrosterone, or pharmaceutically or veterinarily acceptable salts thereof, the dehydroepiandrosterone having the chemical formula



(I);

wherein the broken line represents a single or double bond; R is hydrogen or halogen; the H at position 5 is present in the alpha or beta configuration or the compound of chemical formula I comprises a racemic mixture of both configurations; and R_1 is SO_2OM, wherein M is H

wherein the pharmaceutical composition comprises particles about 15 μm to about *500* μm in size.

188. (Previously Presented) The pharmaceutical composition of claim 187, wherein said active agent is dehydroepiandrosteronesulfate.

189. (Previously Presented) The pharmaceutical composition of claim 187, which is an inhalable or nasal formulation.

190. *(*Previously Presented*)* The pharmaceutical composition of claim 187, further comprising an amount of ubiquinone (CoQn, wherein n=1 to 12) effective to reduce adenosine depletion in an animal tissue.

IX. EVIDENCE APPENDIX

1. Prendergast U.S. Patent 4,956,355
2. Lieberman et al., "Pharmaceutical Dosage Forms", page 110
3. Gennaro, Alfonso R., "Remington: The Science and Practice of Pharmacy", 17^{th} Ed., 1985, page 1505
4. Kelly et al., "Chapter 24/Asthma", 2^{nd} Ed., 1992, pages 408-449
5. Nyce U.S. Patent 5,527,789
6. Lieberman et al., "Pharmaceutical Dosage Forms", pages 32-37
7. Table 1: Partial lists of modes of drug delivery and pharmaceutical and medicinal agents from Genarro, Alfonso R., "Remington's Pharmaceutical Sciences", 17^{th} Ed. 1985

United States Patent [19]

Prendergast

[11] **Patent Number: 4,956,355**

[45] **Date of Patent: Sep. 11, 1990**

[54] **AGENTS FOR THE ARREST AND THERAPY OF RETROVIRAL INFECTIONS**

[75] Inventor: **Patrick T. Prendergast**, Baybush, Ireland

[73] Assignee: **Colthurst Limited, Baybush, Ireland**

[21] Appl. No.: **182,480**

[22] Filed: **Apr. 15, 1988**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 90,637, Aug. 27, 1987, abandoned.

[30] **Foreign Application Priority Data**

Apr. 16, 1987 [IE] Ireland 997/87
Aug. 27, 1987 [IE] Ireland 2289/87

[51] **Int. Cl.**[5] **A61K 31/56; A61K 31/58;** A61K 31/665; A61K 31/66

[52] **U.S. Cl.** **514/178;** 514/99; 514/102; 514/121; 514/172; 514/171

[58] **Field of Search** 514/171, 172, 178, 99, 514/102, 121

[56] **References Cited**

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Henderson, et al., Dehydroepiandrosterone and 16α-bromo-epiandrosterone: Inhibitors of Epstein-Barr Virus-Induced Transformation of Human Lymphocytes, Carcinogenesis, vol. 2, No. 7, 1981, pp. 683–686.

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Ho, et al., Infection of Monocyte/Macrophages by Human T Lymphotropic Virus Type III, The American Society for Clinical Investigation, Inc., vol. 77, May 86, pp. 1712–1715.

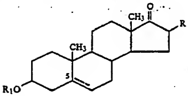
Koo, et al., Effect of Dehydroepiandrosterone on Hereditary Angiodema, Klin Wochenschr, (1983) 61:715–717.

Lucas, et al., Prevention of Autoantibody Formation & Prolonged Survival in New Zealand . . . , The American Society of Clinical Investigations, Inc., vol. 75, Jun. 85, 2091–2093.

Primary Examiner—Joseph A. Lipovsky
Attorney, Agent, or Firm—Marla J. Church

[57] ABSTRACT

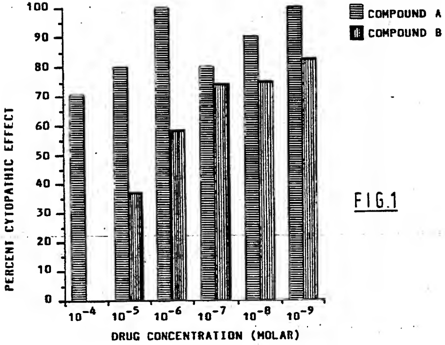
Compounds of the general Formula (I)



(I)

in which R is a hydrogen atom or a bromine atom, and R_1 is a hydrogen atom, an SO_2OM group wherein M is a hydrogen or sodium atom, various sulphatide or phosphatide groups or a glucuronide group are disclosed for use in the prophylaxis and therapy of retroviral infections, especially infection by Human Immunodeficiency Virus. These compounds may be used concomitantly or in combination with various immunomodulators and/or antiviral agents.

16 Claims, 3 Drawing Sheets



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PERCENT CYTOPATHIC EFFECT
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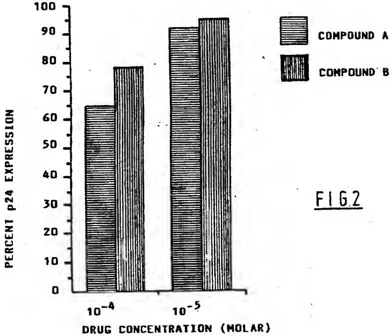
DRUG CONCENTRATION (MOLAR)
COMPOUND A
COMPOUND B

FIG.1



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PERCENT p24 EXPRESSION
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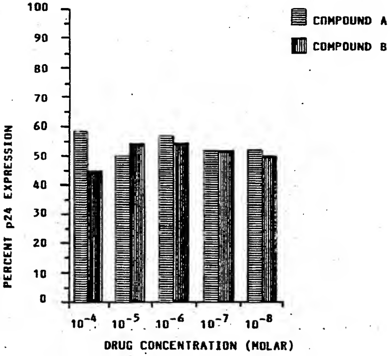
DRUG CONCENTRATION (MOLAR)
COMPOUND A
COMPOUND B

FIG.2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PERCENT p24 EXPRESSION
COMPOUND A
COMPOUND B
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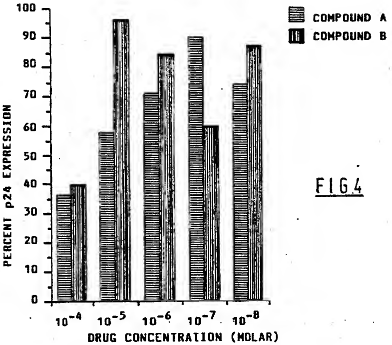
10^-5
10^-6
10^-7
10^-8
DRUG CONCENTRATION (MOLAR)

FIG.3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PERCENT p24 EXPRESSION
COMPOUND A
COMPOUND B
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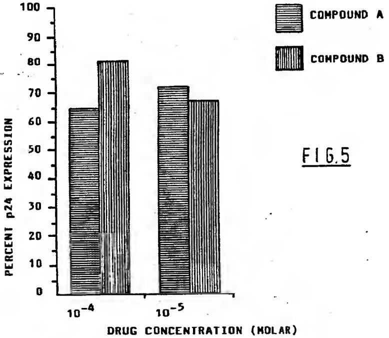
10^{-7}
10^{-8}
DRUG CONCENTRATION (MOLAR)

FIG.4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PERCENT p24 EXPRESSION
COMPOUND A
COMPOUND B
10^{-4}
10^{-5}
DRUG CONCENTRATION (MOLAR)

FIG.5

AGENTS FOR THE ARREST AND THERAPY OF RETROVIRAL INFECTIONS

This case is a continuation-in-part of Ser. No. 07/090,637 Filed 8/27/87; now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to the use of certain 17-ketosteroids in the prophylaxis and therapy of retroviral infections or a complication or consequence thereof. In particular, the invention relates to the use of said 17-ketosteroids in the prophylaxis and therapy of retroviral infections leading to a deficiency of the immune system resulting in the development of opportunistic infections and certain cancers. More especially, the invention relates to the use of the 17-ketosteroids in the prophylaxis and therapy of retroviral infections thought to be responsible for the Acquired Immune Deficiency Syndrome (AIDS) and the related disease AIDS related complex (ARC). AIDS and ARC are believed to result from infection by the Human Immunodeficiency Virus (HIV) and antibodies to which are found in the serum of almost all persons diagnosed as suffering from AIDS or ARC. Lymphadenopathy-associated virus (LAV) and human T-lymphotrophic virus type III (HTLV-III), as well as related retroviruses have been isolated from a large number of AIDS patients. All of these viruses share important characteristics. HTLV-III and LAV are now believed to be strains of the same virus, which has been given the name Human Immunodeficiency Virus (HIV).

AIDS is a disease characterised by loss of cell-mediated immunity and the development of frequent and eventually fatal opportunistic infections. The diagnosis of AIDS is a clinical one, defined as "the occurrence of an illness predictive of a defect in cell-mediated immunity occurring in an individual with no known cause for diminished resistance to that disease" (Lane, H.C. & Fauci, A.S. Ann. Rev. Immunol. 1985, 3, 477-500).

The use of the term HIV embraces the retrovirus HIV-1 or HIV-2 (Human Immunodeficiency Virus Type 1 and Human Immunodeficiency Virus Type 2), which was discovered in 1983. HIV attacks and reduces the numbers of a subset of white blood cells known as T lymphocytes. Expressed on the cell surfaces of these T lymphocytes is a molecule known as CD4, (such cells are also known as T4 cells). Such lymphocytes, most of which are included in what is functionally defined as the helper/inducer subset, constitute the major proportion of mature T cells. Another major subset of T cells express the CD8 molecule on their cell surfaces (such cells are also known as T8 cells). Most of these are classified as suppressor/cytotoxic cells. Normally the T4/T8 ratio is 1.5 to 2.0. In AIDS patients, however, this ratio is inverted due to a decrease in the absolute numbers of T4 cells, with normal numbers of T8 cells usually being preserved.

T4 cells specifically recognise and proliferate in response to antigens that they encounter in the body, at the same time releasing a variety of proteins known as lymphokines that regulate other immune system cells. Upon signaling by T4 cells, B lymphocyte cells recognise antigens and secrete specific antibodies to neutralise or eliminate antigenic bacteria and viruses as they travel through body fluids between cells. Similarly, following signaling from T4 cells, cytotoxic T cells ("T8") become activated to kill cells infected with intracellular pathogens. Furthermore, T4 cells modulate the activities of immune system cells known as natural killer cells and macrophages, which are involved in response to infection and perhaps to incipient malignancies.

A critical and early event in HIV infection involves the virus'attachment, via its envelope glycoprotein, to a receptor on the surface of a susceptible T4 cell, the CD4 molecule. The CD4 molecule at the T4 cell surface appears to distinguish potential target cells from HIV and to act as the receptor molecule that binds the virus and allows infection and subsequent viral replication as well as the cytopathic consequences of viral infection.

The immunodeficiency of AIDS clearly demonstrates the importance of T4 lymphocytes. Because of the loss of these cells, the remaining T lymphocytes from AIDS patients have diminished or absent responses to antigens and show subnormal production of essential immuno-regulatory factors. Due to their decreased numbers and functional capacity, T4 cells are unable to fulfil their necessary role in providing direction for the maturation of B cells and cytotoxic T cells. The ability of AIDS patients to mount antibody reactions to new antigens is severely compromised, though paradoxically high levels of antibodies to previously encountered antigens, including HIV, are often present in patients'sera (Institute of Medicine, National Academy of Science, Confronting AIDS, Washington, D.C. National Academy Press 1986, pages 37-44 and 177-199).

At present AIDS and ARC are predominantly found in certain high risk groups such as homosexuals, intravenous drug abusers and those who have received multiple transfusions or products such as Factor VIII derived from blood. Blood donors are now routinely screened for antibodies to HIV and, therefore, future spread of HIV through blood transfusions and blood-derived products should not, hopefully, lead to transmission of AIDS. AIDS is also increasingly found in the heterosexual population.

There is increasing evidence that macrophage/monocyte infection is a vital factor in the persistence and progession of HIV infection, in initiating the brain damage that occurs in AIDS and in triggering the collapse of the immune system as evidenced by eventual profound depletion of T4 lymphocytes. Crowe et al. have demonstrated using anti-HIV p24 antibody that monocyte/macrophages can be infected with HIV. They have demonstrated that up to 70% of cells from individual donors could be infected (AIDS Research and Human Retroviruses, Vol. 3, No. 2, 1987, page 135). Nicholson et al. have proposed an HTLV-III/LAV-induced effect in monocyte function rather than (or in addition to) an intrinsic defect in surviving T cells to account for observed abnormalities in T cell assays that are monocyte-dependent such as pokeweed mitogen-induced Ig synthesis and proliferative responses to soluble antigens. These T cell assays have previously been reported as abnormal even when assayed as T cell subsets (The Journal of Immunology, Vol. 137, No. 1, 1986, page 323).

Since it is well established that the first event that occurs when a foreign material (for example, a virus) enters the body is its uptake by mononuclear phagocytes, it is conceivable that these cells represent a primary target for HIV. Gartner et al. have shown that virus production by HTLV-III/LAV infected macrophages was high and long-lived, indicating that these cells may play a role in virus dissemination and persis-

tence. They have demonstrated HTLV-III/LAV replication in macrophages was fully productive in the situations they evaluated (Science Vol. 233, 1986, page 215).

Salahuddin et al. observed that in vitro pulmonary macrophages can be infected with HTLV-III and appear to be less susceptible to the phytopathic effects of this retrovirus which suggests that tissue macrophages should be considered as potential reservoirs of HTLV-III in vivo (Blood, Vol. 68, No.1, 1986, page 281).

Ho D.D. et al. observed normal blood-derived monocytes/macrophages were found to be susceptible to infection in vitro by human T Lymphotropic virus III (HTLV-III), the etiologic agent of the Acquired Immune Deficiency Syndrome. In addition, HTLV-III was recovered from monocytes/macrophages of patients infected with this virus. It was postulated therefore that HTLV-III-infected monocyte/macrophages may serve as a vehicle for the dissemination of virus to target organs and as a reservoir for viral persistence, as has been shown for other lentiviruses, including visna virus and caprine arthritis encephalitis virus (J. Clin. Invest., Vol. 77, 1986, page 1712).

While an antiviral agent which could kill all infecting HIV or completely inhibit its replication (and at the same time have an acceptable toxicity profile) is clearly desirable, the situation is that no such agent is at present available.

With the emerging understanding of the role that macrophages may be playing in the pathogenesis of AIDS, it is clear that an effective antiviral strategy will require an approach that can treat infected macrophages and inhibit infection of these cells. Currently the only F.D.A. approved antiviral agents for treatment of AIDS are azido thymidine (AZT) and pentamidine isethionate (PENTAM 300). As demonstrated hereinafter AZT is completely ineffective at inhibiting macrophage infection or modulating HIV production from infected macrophages. Administration of AZT over long periods of time has been found to give rise to undesirable side effects such as anaemia, necessitating blood transfusion, leucopenia and neutropenia.

The great majority of antiviral compounds are nucleosides, including, for example, AZT.

Many of the 17-ketosteroids function as hormones and include sex hormones or precursors thereof and hormones which control metabolism. Dehydroepiandrosterone (DHEA) is one such 17-ketosteroid which is a precursor of both androgens and estrogens and additionally has important metabolic effects. These effects ensue from its inhibitory effect on enzymes such as glucose-6-phosphate dehydrogenase and NADH oxidase. Additionally, DHEA has an inhibitory effect on mitotic activity and on the permeability of membranes (Jiri Sonka, Acta Universitatis Carolinae Medica Monographia LXXI -1976). The effect of DHEA on enzymes such as glucose-6-phosphate dehydrogenase and NADH oxidase leads above all to inhibition of the pentose cycle and of the cytochrome system, both of which restrict the supply of building materials and energy, necessary for biosynthetic processes, in particular for growth and regeneration of tissue. One of the main conditions of growth is an adequate supply of energy (ATP) and building materials for nucleic acid synthesis. DHEA controls both of these processes as an inhibitor of NADH oxidase and glucose-6-phosphate dehydrogenase. DHEA has been found to suppress some of the metabolic disorders and liver cirrhosis, and reduces pain in ischemic heart disease, especially in angina pectoris, by restricting tissue respiration. HEA has been used in the treatment of menopause, emotional instability, depression and stress.

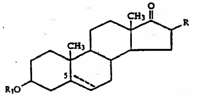
Individuals who are genetically deficient in glucose-6-phosphate dehydrogenase are relatively resistant to Falciparum Malaria and have much smaller numbers of protozoa in their erythrocytes than normal individuals (Motulski, A.G. 1975, in "The Role of Natural Selection in Human Evolution", Ed. Salzano, S. Amsterdam, New Holland, P.271 and Luzzato, L. et al., Science, 164, 839, 1969).

DHEA and related compounds are capable of reducing the colony forming ability of human peripheral blood mononuclear (PBM) cells infected with Epstein-Barr virus (a herpes virus) at concentrations of 10-100 μM (Carcinogenesis, Vol. 2, pp 883–886, 1981).

DHEA also inhibits complement activation and is therefore of value in the prophylaxis of Hereditary Angioneurotic Oedema (Hidvegi et al., Complement 1; 201, 1984). DHEA also prevents autoantibody formation in the murine model of Systemic Lupis Erythematosus (SLE) and many of the features of full-blown AIDS are considered to be similar to those of SLE (Lucas et al., J. Clin. Invest., 75: 2091, 1985).

SUMMARY OF THE INVENTION

According to the invention there is provided a compound for use in the prophylaxis and therapy of a retroviral infection, or a complication or consequence thereof, the compound having the general formula (I)



(I)

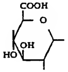
in which R is a hydrogen or bromine atom, and R_1 is a hydrogen atom, an SO_2OM group wherein M is a hydrogen or sodium atom, a sulphatide group

$$-SO_2O-CH_2.\underset{\displaystyle O.CO.R_2}{CH}.CH_2O.CO.R_3$$

a phosphatide group

$$-\overset{\displaystyle O}{\underset{\displaystyle O}{\overset{\|}{\underset{\|}{P}}}}-O.CH_2.\underset{\displaystyle O.CO.R_2}{CH}.CH_2.O.CO.R_3$$

wherein each of R_2 and R_3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



wherein the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the α- or β- configuration or the compound comprises a mixture of both configurations.

When R_1 is other than a hydrogen atom, the compounds are conjugated compounds.

Preferably in the compound of formula (I), R and R_1 are each hydrogen. An especially preferred compound is dehydroepiandrosterone wherein R and R_1 are each hydrogen and the double bond is present.

In a further embodiment of the invention, the compound is 16α-bromoepiandrosterone, wherein R is Br, R_1 is H and the double bond is present. In a still further embodiment of the invention, the compound is etiocholanolone wherein R and R_1 are each hydrogen and the double bond is absent.

Other preferred compounds are dehydroepiandrosterone sulphate, wherein R is H, R_1 is SO_2.OM and M is as hereinbefore defined and the double bond is present, and 5β-androstan- 3β-ol-17-one.

Alternatively, the compound is selected from dehydroepiandrosterone sulphatides, phosphatides or glucuronide wherein R is H, and R_1 is a sulphatide, phosphatide or glucuronide group as hereinabove defined, and the double bond is present.

Additionally, the invention provides a pharmaceutical formulation for use in the prophylaxis and therapy of a retroviral infection or a complication or consequence thereof, comprising a prophylactically or therapeutically effective amount of at least one compound of the formula (I) as an active ingredient.

The pharmaceutical formulation according to the invention may be administered locally or systemically. By systemic administration is meant any mode or route of administration which results in effective levels of active ingredient appearing in the blood or at a site remote from the site of administration of said active ingredient.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, dragees, pills, tablets, including coated tablets, elixrs, suspensions, syrups or inhalations and controlled release forms thereof.

Solid dosage forms in addition to those formulated for oral administration include rectal suppositories.

The compound of the formula (I) may also be administered in the form of an implant.

Suitable formulations for topical administration include creams, gels, jellies, mucilages, pastes and ointments. The compounds may also be formulated for transdermal administration, for example, in the form of transdermal patches so as to achieve systemic administration.

Suitable injectable solutions include intravenous, subcutaneous and intramuscular injectable solutions. The compound of the formula (I) may also be administered in the form of an infusion solution or as a nasal inhalation or spray.

The pharmaceutical formulation according to the invention is administered in unit doses comprising from to 1,000 mg of active ingredient. Preferably, each unit dose comprises from 50 to 500 mg of active ingredient.

According to one embodiment of the invention, the compound of formula (I) is administered at a rate of from 1 unit dose to 10 unit doses per day Administration of the compound of the formula (I) in accordance with the invention is continued for a period of at least five days and in certain cases may be given for the life of the individual.

Further, the invention provides use of a compound of the formula (I) in the manufacture of a medicament for use in the prophylaxis or therapy of a retroviral infection, or a complication or consequence thereof.

The invention also provides a method for treating a retroviral infection in a human or non-human patient, comprising administering a therapeutically effective amount of a pharmaceutical formulation comprising a compound of the formula (I) to said patient.

The invention further provides a method for the prophylaxis of a retroviral infection in a human or non-human patient, comprising administering a prophylactically effective amount of a pharmaceutical formulation comprising a compound of the formula (I) to said patient.

The compounds of the formula (I) hereinabove given and defined are particularly useful for the prophylaxis and therapy of infection by HIV, or a complication or consequence thereof.

According to a further aspect of the invention there is provided a method for the prophylaxis and therapy of Acquired Immunodeficiency Syndrome (AIDS) in a patient, which comprises administering to said patient a prophylactically or therapeutically effective amount of a compound of the formula (I) or a pharmaceutical formulation containing it.

According to a still further aspect of the invention, there is provided a method for the prophylaxis and therapy of Acquired Immunodeficiency Syndrome Related Complex (ARC) in a patient, which comprises administering to said patient a prophylactically or therapeutically effective amount of a compound of the formula (I) or a pharmaceutical formulation containing it.

The compounds of the formula (I) may also be used concomitantly or in combination with an immune system booster or immunomodulator as an agent in the prophylaxis and therapy of a retroviral infection, or a complication or consequence thereof. In this way the immunomodulator booster may be used to enhance the production of T-cells by the bone marrow.

The immunomodulator may be administered prior to and in an amount sufficient to stabilise or increase the production of T-cells prior to administering said compound of the formula (I). In particular, the immunomodulator is administered until the rate of production of T-4 cells is stabilised or begins to increase.

The compound of the formula (I) and the immunomodulator may be combined in a single dosage form or in discrete dosage forms.

Suitable immune system boosters or immunomodulators for use in accordance with the invention are selected from ABPP (Bropirimine); Ampligen (mismatched RNA) developed by Du Pont/HEM Research; anti-human α-Interferon antibody manufactured by Advance Biotherapy and Concepts; anti-AIDS antibody (Nisshon Food); AS-101 (heavy metal based immunostimulant), ascorbic acid and derivatives thereof; β-interferon; Carrosyn (polymannoacetate); Ciamexon manufactured by Boehringer Mannheim; Cyclosporin; Cimetidine; CL246,738 manufactured by American Cyanamid; colony stimulating factor (CM-CSF) manu-

factured by Sandoz and Genetics Institute; dinitrochlorobenzene (DNCB); α-interferon; γ-interferon; glucan; Hyperimmue (gamma-globulin) manufactured by Bayer; IMREG-1 (leucocyte dialyzate) and IMREG-2 manufactured by IMREG; immuthiol (sodium diethylthiocarbarmate) manufactured by Institut Merieux; Interleukin-1, Interleukin-2 manufactured by Cetus Corporation, Hoffmann-La Roche and Immunex; isoprinosine (inosine pranobex); Krestin manufactured by Sankyo; LC-9018 developed by Yakult; Lentinan manufactured by Ajinomoto/Yamanouchi; LF-1695 manufactured by Fournier; MET-ENK (methionine-enkephalin) manufactured by TNI Pharmaceuticals and Sygma Chemicals; Minophagen C; MTP-PE (muramyl tripeptide) manufactured by Ciba-Geigy; Trexan (Naltrexone) manufactured by Du Pont; Neutropin; RNA immunomodulator developed by Nippon Shingaku; shosaikoto and ginseng; thymic humoral factor; TP-5 (Thymopentin) manufactured by Ortho Pharmaceuticals; Thymosin fraction 5 and Thymosin 1; Thymostimulin; TNF (tumor necrosis factor) manufactured by Genentech; and vitamin B preparations.

The majority of the above mentioned immunomodulators are administered orally. Dinitrochlorobenzene is normally applied topically by painting onto the skin of the patient.

Accordingly, the invention also provides a pharmaceutical formulation comprising a compound of the formula (I) together with an effective amount of an immune system booster or immunomodulator.

The invention also provides a compound of the formula (I) for use concomitantly or in combination with an antiviral agent in the prophylaxis and therapy of a retroviral infection, or a complication or consequence thereof.

The compound of the formula (I) and the antiviral agent may be combined in a single dosage form or in discrete dosage forms.

Suitable antiviral agents include AL-721 (lipid mixture) manufactured by Ethigen Corporation and Matrix Research Laboratories; Amphotericin B methyl ester; Ampligen (mismatched RNA) developed by Du Pont-/HEM Research; anti-AIDS antibody (Nisshon Food); AS-101 (heavy metal based immunostimulant); AZT (azidothymidine/Retrovir/Zidovudine) manufactured by Burroughs Wellcome; Betaseron (β-interferon) manufactured by Triton Biosciences (Shell Oil); butylated hydroxytoluene; Carrosyn (polymannoacetate) Castanospermine; Contracan (stearic acid derivative); Creme Pharmatex (contains benzalkonium chloride) manufactured by Pharmelac; CS-87 (5-unsubstituted derivative of Zidovudine); Cytovene (ganciclovir) manufactured by Syntex Corporation; DDC (dideoxycytidine) manufactured by Hoffmann-La Roche and other nucleoside analogues; dextran sulphate; D-penicillamine (3-mercapto-D-valine) manufactured by Carter-Wallis and Degussa Pharmaceutical; Foscarnet (trisodium phosphonoformate) manufactured by Astra AB; fusidic acid manufactured by Leo Lovens; glycyrrhizin (a constituent of liquorice root); HPA-23 (ammonium-21-tungsto-9-antimonate) manufactured by Rhone-Poulenc Sante; human immunevirus antiviral developed by Porton Products International; Ornidyl (eflornithine) manufactured by Merrell Dow; Nonoxinol; pentamidine isethionate (PENTAM 300) manufactured by Lypho Med; Peptide T (octapeptide sequence) manufactured by Peninsula Laboratories; Phenytoin marketed by Park-Davis (Warner-Lambert Company); Ribavirin; Rifabutin (ansamycin) manufactured by Adria Laboratories; rsT4 (recombinant soluble T4) manufactured by Biogen, Genentech and Smith Kline & French; Trimetrexate manufactured by Warner-Lambert Company; SK-818 (germanium-derived antiviral) manufactured by Sanwa Kagaku; suramin and analogues thereof manufactured by Miles Pharmaceuticals; UA001 manufactured by Ueno Fine Chemicals Industry; Wellferon (α-interferon) manufactured by Burroughs Wellcome; Zovirex (acyclovir) manufactured by Burroughs Wellcome.

It will be observed that the above mentioned antiviral agents include some of the agents hereinbefore specified for use as immunomodulators together with a compound of the formula (I) in accordance with the invention. Isoprinosine, for example, is known to act as an immunomodulator but also has antiviral properties. The term "antiviral" as used in the present Specification also include agents which interfere with the entry of retroviruses into a cell.

The invention may also provide a compound of the formula (I) for use concomitantly or in combination with a drug useful in the prophylaxis and therapy of AIDS-associated opportunistic infections.

As indicated hereinafter the compounds of formula (I) are particularly suitable for use as inhibitors of retroviruses, especially HIV, in macrophages.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following drawings:

FIG. 1 is a schematic representation of percent cytopathic effect versus drug concentration in the VB tumor cell line for two compounds, designated A and B, used in accordance with the invention;

FIG. 2 is a schematic representation of percent p24 expression versus drug concentration in activated peripheral blood lymphocytes for two compounds, designated A and B, used in accordance with the invention;

FIG. 3 is a schematic representation of percent p24 expression versus drug concentration in human macrophages for two compounds, designated A and B, used in accordance with the invention;

FIG. 4 is a schematic representation of percent p24 expression versus drug concentration in HIV infected macrophages for two compounds, designated A and B, used in accordance with the invention; and

FIG. 5 is a schematic representation of percent p24 expression versus drug concentration for chronically infected human macrophages for two compounds, designated A and B, used in accordance with the invention.

EXAMPLES

The invention will be further illustrated by the following Examples.

In the following Examples 1 to 7 compound A corresponds to dehydroepiandrosterone and compound B corresponds to 16α-bromoepiandrosterone.

In relation to Examples 1 to 7, the materials used and the analyses and assays carried out were as follows:

1. Source of HIV: To analyse antiviral effects in Examples 1 to 7 three substrains of HIV were used: the HTLV-IIIB strain of HIV, currently grown in tissue culture in the H9 cell line; the low passage isolate HIV-DV, a strain which has been shown to infect human macrophages; and the ARV-2 HIV strain first isolated by Dr. Jay Levy in San Francisco. All three viral strains were grown in the Tissue Culture Laboratory of the AIDS Activity Division of San Francisco General Hos-

pital where titers of 10^{-4} to 10^{-6} infectious units per ml were routinely achieved.

2. Source of cells: The VB cell line used in Example 1 is a T lymphoma cell line that is highly susceptible to infection with HIV, and expresses high levels of cell surface CD4 molecules. This cell line forms syncytia within two days after infection with all strains of HIV tested to date. The H9 cell line is a T cell ALL that is susceptible to infection with virtually all strains of HIV, however, does not form syncytial cells. This is used to quantitate infection in the absence of syncytial formation, infection being quantitated by immunofluorescence assays. The HXB/H9 cell line (Example 6) is an H9 cell population that chronically produces the HTLV-IIIB strain of HIV and is utilised in experiments testing antiviral effects on chronically infected cell lines. Human macrophages were prepared from peripheral blood mononuclear cells either obtained from the blood bank as a buffy coat, or as a leukophoresis preparation. Crowe et al. supra have devised an assay system that allows quantitation of HIV infection, and inhibition of infection using immunocytofluorographic analysis. In order to quantitate HIV infection in macrophages, they grow macrophages in Teflon (Trade Mark) culture vessels which maintain macrophages in suspension in vitro culture for up to six months. Cell surface and cytoplasmic immunofluorescence staining is then performed to quantitate antigens in macrophages by flow cytometry.

3. Flow cytometry: An Ortho Cytofluorgraf II-S (Trade Mark) that has a biohazard containment flow cell was used for analysis of HIV infected samples. HIV p24 antigens were detected utilizing a mouse monoclonal anti-p24 (du Pont), and mouse antibodies were identified utilizing an FITC conjugated goat anti-mouse IgG.

4. HIV soluble p24 antigen detection: Soluble p24 antigens were measured with the Abbott HIV antigen detection system.

5. Inhibition of acute infection: Several assays were utilized to test for inhibition of acute infection; these included:

a) Inhibition of multinucleated giant cell formation in acutely infected VB cells infected at a multiplicity of infection of 1, and scored two days after infection in the presence or absence of varying concentrations of drug, for the formation of multinucleated giant cells. (free virus is washed out after a one hour incubation pretreatment at room temperature). Monoclonal antibody anti-Leu3a completely inhibits the formation of syncytial cells, and was utilized as a positive control for infection inhibition. Supernatants were also isolated and the level of HIV p24 antigen determined. Infectious virus was measured in treated cultures by performing a syncytial assay as described above.

b) Although the VB T lymphoma cell line behaves in a similar manner to peripheral blood CD4 positive T lymphocytes in regard to HIV induced cytopathic effects, the above described experiments were performed on phytohemagglutinin (PHA) activated lymphocytes to determine whether lymphocytes are more or less sensitive to Compounds A and B than is the VB cell line. In this assay system, at the time that multinucleated giant cells appeared within infected lymphocyte cultures, the supernatants were analyzed for the presence of HIV p24 antigens and were titered on indicator VB lymphoma cells to determine the titer of infectious virus present at each point.

c) To test whether Compounds A and B were effective at blocking acute infection of macrophages, macrophages from Teflon cultures were exposed at a multiplicity of 1 to HIV-DV in the presence or absence of various concentrations of drug (Examples 3–5). Normally, HIV expression peaks in human macrophages at approximately ten days after initial infection. Therefore, after infection for one hour at room temperature, followed by a wash and resuspension of the macrophages in various drug concentrations, macrophages were stained for the presence of p24 antigen at day ten. Culture supernatants from these macrophages were assayed for the presence of soluble p24, and infectious virus as described above.

6. Analysis of chronically infected cells: To determine whether compounds A and B are effective at inhibiting HIV expression in macrophages and chronically infected T cells, the following experiments were performed. The chronically infected T cell line, HXB/H9 was exposed to various concentrations of drug for four days in vitro. At four days, the HXB cells were assayed for the presence of p24 intracytoplasmically, and supernatants were assayed for the presence of infectious virus as described above. These same experiments were performed on macrophages that had been infected in vitro and had been shown to be chronically infected by cytofluorographic analysis.

7. Analysis of nonspecific toxicity to the target cells with Compounds A and B. The VB, H9 and HXB cell lines were exposed to different concentrations of Compounds A and B as were normal human macrophages for the length of time the drug was in contact with each cell line as described in the above assays. Cell numbers were counted, and live versus dead were determined by Trypan blue exclusion assays. These tests were required to determine a therapeutic index between nonspecific toxic effects on the described cells, compared with potential effective antiviral effects in vitro (Example 6).

EXAMPLE 1

Inhibition of HIV mediated cytopathic effects in the VB tumor cell line

Compounds A and B were tested for inhibition of T lymphoma cell cytopathic effect at various drug concentrations after acute infection with HIV for 48 hours. FIG. 1 indicates the percent cytopathic effect observed in cultures exposed to various concentrations of compounds A and B. It will be observed that compound B appears to be more active at inhibiting HIV mediated multinucleated syncytial cell formation than compound A. The values for cytopathic effect shown in FIG. 1 were obtained for an average of two experiments. Two subsequent experiments utilizing compounds A and B that had been diluted in dimethyl sulphoxide (DMSO) revealed a less striking effect. It is possible that the diminished effects noted in later experiments could have been secondary to drug stability problems. At 10^{-4} molar compound B appeared to be extremely toxic to the VB T lymphoma cell line, a characteristic not shared by either the normal peripheral blood lymphocytes or macrophages exposed to 10^{-4} molar compound B, as hereinafter indicated in Examples 2 and 3, respectively.

EXAMPLE 2

Inhibition of HIV mediated cytophatic effects in activated peripheral blood lymphocytes

To test whether the effects of compounds A and B on acute VB T lymphoma infection would be mimicked by activated peripheral blood lymphocytes (PBC), HIV at a multiplicity of infection of one was added to lymphocytes that had been activated for 48 hours with 2 μg per ml of PHA. After the initial infection for one hour, the activated lymphocytes were washed and resuspended for the on week of culture. Multinucleated giant cells began to form approximately 7 days after the initial infection, at which time the culture supernatants were harvested and tested for the presence of HIV p24 antigens. The accumulation of HIV p24 antigens in the supernatant is representative of production of HIV from infected cells. It will be noted from FIG. 2 that p24 antigen production was moderately inhibited at 10^{-4} molar with both compounds A and B and was slightly less inhibited at 10^{-5} molar. No appreciable toxicity was noticed at either drug concentration with the PBL cultures.

EXAMPLE 3

Inhibition of HIV production in normal human macrophages

To test whether compounds A and B might be active at inhibiting infection of normal human macrophages, a spectrum of drug concentrations were tested for inhibition of acute infection, and inhibition of HIV expression in chronically infected macrophages. FIG. 3 indicates the presence of HIV p24 antigens in the supernatant of macrophages infected, washed and allowed to become productively infected for one week. After acute infection (one hour) cells were incubated in various concentrations of compounds A and B, and 7 days after the initial infection supernatants were harvested for p24 antigen quantitation. It will be observed that over a very broad range of drug concentration (10^{-4} through 10^{-8} molar) there appeared to be a substantial, approximately 50% decrease in production of HIV p24 antigens. Macrophages treated with DMSO (at 10^{-4} molar drug concentration the final DMSO concentration was 0.05%) at concentrations required to dissolve compounds A and B showed no effect, therefore, these effects were apparently secondary to actions of compounds A and B.

EXAMPLE 4

Inhibition of HIV p24 antigen in HIV-infected macrophages

The cells from the experiment described in Example 3 were analysed, and the cytoplasm was analysed for the presence of HIV p24 to directly test whether HIV p24 antigen production was inhibited within those infected cells. FIG. 4 indicates a composite of three separate experiments utilizing infected macrophages from three different donors. It will be observed that HIV p24 cytoplasmic antigen production was substantially inhibited at 10^{-4} molar with both drugs, and that compound A was active at dilutions even at 10^{-6} molar in inhibiting HIV p24 antigen production within infected macrophages. Therefore, the decrease in HIV p24 within infected supernatants appeared to be associated with decreased HIV p24 antigen production within the infected macrophages.

EXAMPLE 5 (COMPARISON)

The same experiments described in Example 4 were repeated with AZT, at concentrations from 0.05 μg per ml to 50 μg per ml with no change from control values. Accordingly, the inhibition of HIV p24 antigen appeared to be specific at least in this test, for compounds A and B and was not a characteristic of AZT even at very high doses.

EXAMPLE 6

Antiviral testing of chronically HIV infected cell line, HXB

Chronically HIV infected cell line, HXB was tested with compounds A and B for antiviral effects, and other than killing with compound B at concentrations of 10^{-4} molar there appeared to be no specific inhibition of HIV production of cytopathic effects in the chronically infected lymphoma cell line.

EXAMPLE 7

Antiviral testing of chronically HIV infected macrophages

Because macrophages can be infected and produce HIV for very long periods of time without substantial loss of viability, chronically infected cells were tested, specifically a population that was between 30 and 50% HIV antigen positive, for inhibition of cytoplasmic HIV p24 antigen production. FIG. 5 indicates the results of three separate experiments carried out. It will be observed there was some inhibition of HIV p24 antigen production at both 10^{-4} and 10^{-5} molar of compounds A and B, although somewhat less than the inhibition of acute infection of macrophages (FIG. 4). These data suggest that stably infected and chronically producing macrophages may be somewhat inhibited in their HIV p24 antigen production in the presence of compound A and B. Toxicity studies

In all of the experiments described in Examples 1 to 7, the only appreciable toxicity was to tumor cell lines by compound B at 10^{-4} molar. There was not appreciable toxicity in normal macrophages exposed to 10^{-4} to 10^{-8} molar compounds A and B, nor was there appreciable toxicity to peripheral blood lymphocytes exposed to those same levels of drug.

Conclusions

The data presented in Examples 1 to 7, above, are consistent with the following interpretations.

1. Compounds A and B appear to exert a mild antiviral effect in acute infection studies of both T lymphoma cells as well as lymphocytes. In comparison with AZT, compounds A and B are inferior in terms of their antiviral effects, as AZT gives virtually complete protection of both lymphocytes and the T lymphoma cells from acute infection with HIV (as measured at one week) in the range of 1 μg of AZT per ml of culture medium.

2. Of more significance than the antiviral effect noted on the T lymphoma cell line and the peripheral blood lymphocytes were the observed effects of compounds A and B on HIV infection of macrophages. The results obtained in Examples 3–7 are significant, certainly at the level of in vitro inhibition of HIV infection of macrophages and inhibition of macrophage production of HIV. These were reproducible findings that were repeated with six separate monocyte/macrophage do-

nors. Inhibition of HIV infection of macrophage is to date a relatively unique characteristic for an antiviral agent. The fact that compounds A and B inhibit HIV infection and HIV expression in macrophages suggest that they should prove useful for the treatment of HIV infected individuals.

EXAMPLE 8

Use of dehydroepiandrosterone sulphate in AIDS therapy

Dehydroepiandrosterone sulphate was measured into unit doses of 300 mg and 100 mg and each unit dose enclosed in a soft gelatin capsule.

A) A patient sero positive for HIV and diagnosed as suffering from AIDS was treated as follows. For twelve consecutive days, the patient was treated by administering the encapsulated compound orally to the patient. For the first eleven days, a unit dose of 300 mg was administered, once per day. On the twelfth day, the patient was given a single unit dose of 100 mg of the compound.

B) Trials were carried out over a twenty-six day period on two patients sero positive for HIV and diagnosed as suffering from AIDS using the twelve day treatment method discussed in the preceding paragraph except that treatment did not commence until day 5.

Blood samples were taken from the patients on five occasions over the twenty-six day period. The first tests were carried out on each patient on day 1 and included measurements of T1, T4 and T8 cell counts, as well as, sedimentation rate. The treatment commenced on day 5, and continued to day 17. From day 5 to day 16, each patient, as already discussed, received a single unit dose of 300 mg of dehydroepiandrosterone sulphate orally and on day 17 100 mg was administered. The aforementioned tests were repeated on each patient on day 9, day 17, day 24 and day 26. The T4 cell count in each of patients X and Y was found to stabilise as a result of the treatment.

Additionally, while the dehydroepiandrosterone sulphate was being administered orally to the patients, lesions around their mouth and on other parts of their body were treated topically with a cream containing dehydroepiandrosterone sulphate. It was found that the lesions cleared up.

EXAMPLE 9

Use of dehydroepiandrosterone in AIDS therapy

Twelve patients all of whom were sero-positive for HIV and had been diagnosed as suffering from AIDS were treated with DHEA for up to six months. The DHEA was administered in the form of hard gelatin capsules containing 100 mg of DHEA and at a rate of 100 mg to 600 mg per day. The vast majority being on 500 mg per day in divided doses. The patients were all homosexual or bisexual males with an average age of 34.5 years and an average weight of 69.6 kg.

Past and present HIV clinical manifestations included: unexplained diarrhoea, Kaposis Sarcoma, Herpes Zoster, Oral Candidiasis, Lymphadenopathy, Oral Hairy Leucoplakia, involuntary weight loss, dermal mycoses and Stapholococcal skin infections.

All of the patients were in an advanced stage of AIDS at the commencement of the trial and normally further deterioration of their condition, or even death, would have been expected over the six month period of the trial. However, no serious deterioration in condition was observed in any of the patients, with four patients actually gaining weight. Patient 7 gained 8 kg over five months.

Compounds of the formula (I) and, in particular, dehydroepiandrosterone and the derivatives thereof hereinbefore mentioned have particular advantages in the treatment of patients infected with HIV. Particular advantages of such compounds include the virtual absence of toxicity, ease of administration and the unique action on the macrophage system referred to above.

Dehydroepiandrosterone has demonstrated a complete lack of adverse physical, biochemical or hematological effects in twelve subjects who received daily doses of up to 600 mg for up to six months.

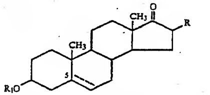
Because of the unique action of dehydroepiandrosterone in the macrophage system, it is possible that use thereof could extend the mean survival time of HIV infected individuals, which is calculated at present to be 8.3 years from the time of infection.

Furthermore, compounds of the formula (I) can be used in synergistic combination with other antiviral agents as indicated above.

Although not wishing to be bound by any theoretical explanation of the invention it is postulated that since dehydroepiandrosterone inhibits glucose-6-phosphate dehydrogenase leading to a depletion of the cellular pool of NADPH, resulting small changes of protein biosynthesis in the cell may, because of the complex regulation of HIV gene expression, lead to significant changes in viral protein production. An example of such a viral regulatory protein is art/trs which is present in very small amounts in infected cells, but is responsible for regulating viral RNA splicing and, consequently, viral protein production.

I claim:

1. "A method for treating or arresting the progression of a retroviral infection in a patient in need of such treatment which comprises administering to said patent A". therapeutically effective amount of a compound of the formula (I).



(I)

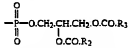
in which R is selected from the group consisting of a hydrogen atom and a bromine atom, and R_1 is a chemical group selected from the group consisting of a hydrogen atom, an SO_2OM group wherein M is selected from the group consisting of a hydrogen atom, a sodium atom, a sulphatide group

$$-SO_2O-CH_2.\underset{\displaystyle O.CO.R_2}{\underset{|}{CH}}.CH_2.O.CO.R_3$$

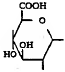
$$-SO_2O-CH_2.\underset{\displaystyle O.CO.R_2}{\underset{|}{CH}}.CH_2O.CO.R_3$$

wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of

straight and branched chain alkyl radicals of 1 to 14 carbon atoms, a phosphatide group



wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radicals of 1 to 14 carbon atoms, and a glucoronide group



wherein the broken line represents an optical double bond, and the hydrogen atom at position 5 is present in the α- or β-configuration or a mixture of both configurations.

2. A method according to claim wherein in the compound of the formula (I) R and R_1 are each hydrogen.

3. A method according to claim 2, wherein the compound is dehydroepiandrosterone, the compound wherein R and R_1 are each hydrogen and the double bond is present.

4. A method according to claim 1, wherein the compound is 16α-bromoepiandrosterone, the compound wherein R is bromine, R_1 is hydrogen and the double bond is present.

5. A method according to claim 1, wherein the compound is dehydroepiandrosterone sulphate.

6. A method according to claim 1, wherein the compound of formula (I) is formulated for systemic administration.

7. A method according to claim 1, wherein the compound of the formula (I) is administered concomitantly or in combination with an immunomodulator.

8. A method according to claim 1, wherein the compound of the general formula (I) is administered concomitantly or in combination with an antiviral agent.

9. "A method according to claim 1 wherein the retroviral infection is a HUman Immunodeficiency Virus Infection".

10. "A method according to claim 1 wherein the retroviral infection has progressed to Acquired Immunodeficiency Syndrome (AIDS)."

11. A method according to claim 10, wherein the compound of the Formula (I) is administered concomitantly, or in combination with an immunomodulator.

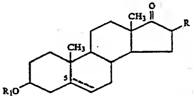
12. A method according to claim 10, wherein the compound of the Formula (I) is administered concomitantly or in combination with an antiviral agent.

13. "A method according to claim 1 wherein the retroviral infection has progressed to Acquired Immunodeficiency Syndrome Related Complex (ARC).

14. A method according to claim 13, wherein the compound of Formula (I) is administered concomitantly or in combination with an immunomodulator.

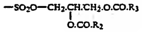
15. A method according to claim 13, wherein the compound of the Formula (I) is administered concomitantly or in combination with an antiviral agent.

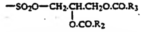
16. A method for the treatment of a patient who has a retroviral infection, which comprises administering to said patient a prophylactically or therapeutically effective amount of a compound of the formula (I)



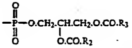
(I)

in which R is selected from the group consisting of a hydrogen atom and a bromine atom, and R_1 is a chemical group selected from the group consisting of a hydrogen atom, an SO_2OM group wherein M is selected from the group consisting of a hydrogen atom, a sodium atom, a sulphatide group

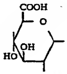




wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radicals of 1 to 14 carbon atoms,
a phosphatide group



wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radicals of 1 to 14 carbon atoms,
and a glucuronide group



wherein the broken line represents an optical double bond, and the hydrogen atom at position 5 is present in the α-or β- configuration or a mixture of both configurations.—

* * * * *

PHARMACEUTICAL DOSAGE FORMS

Tablets

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3. Friction of the particles colliding with each other
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A considerable research expenditure has been made in developing theoretical and practical approaches to improve efficiency [14] and to solve the problems of scale-up milling from the laboratory to production. This expenditure was being carried primarily by "heavy industry," namely the coal, coke, ore (steel included), cement, and paint industries. Because of the enormous volume of materials handled in these industries (tons to thousands of tons per hour), the scale-up, purchase, fabrication, and installation of equipment that handle these material volumes—and withstand the loads and wear encountered—constitute major investments for the corporations involved. With the exception of a number of continuous pharmaceutical manufacturing processes now in operation, pharmaceutical production does not approach the production scale encountered in "heavy industry." This is the primary reason why the pharmaceutical industry is not oriented toward designing and testing new size reduction equipment. A number of smaller equipment companies appear to be meeting the pharmaceutical industries' needs by getting into the size reduction of biological products, and have merely extended their line to include the size reduction of solids. Size reduction and scale-up problems in the pharmaceutical industry are very similar to those found in "heavy industry," and are more often solved empirically rather than through the theoretical route. There is some application of mathematics developed for specific size reduction equipment, and this will be discussed in Sec. II, Size Reduction Equipment.

Size reduction is nothing new to pharmacy, as evidenced by the array of age-old metal, wooden, and ceramic mortars and pestles that have been preserved from antiquity and which were used in the early apothecaries in the preparation of powder mixes, pills, and plant and animal extracts.

Size reduction, as it applies to tablet production, falls into three basic categories, namely: (a) the reduction in size of oversized and/or agglomerated raw materials, (b) the reduction in size of wet and dry granular materials usually of multi-ingredient compositions, and (c) the reduction in size of tablets or compactions which must be milled for dry granulating or reworking.

Size reduction and the use of size reduction equipment creates certain advantages in tablet formula development and their subsequent production, including:

1. Increase in surface area, which may enhance an active ingredient's dissolution rate and hence, its bioavailability. This is particularly important with compounds that are slightly soluble, such as phenacetin. The affect in phenacetin dissolution rate and bioavailability as a result of small particle size differences is illustrated in Figure 2. Improved bioavailability with improved dissolution rate has been demonstrated by Ullah et al. [16]. Effects on pharmaceuticals has been well documented by E. L. Parrott [17]. It must be noted, however, that active ingredients reduced in particle size to gain the advantage of increased surface area, may not retain all of this advantage after being incorporated into a wet or dry granulation mix, and compressed into tablets.
2. Improved tablet to tablet content uniformity by virtue of the increased number of particles per unit weight. The more particles

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Pharmaceutical

Sciences

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carboxymethylcellulose or similar substances) contain saccharin. During the past few years, scientists have been studying the toxic effects of this sweetening agent and of the cyclamates. The cyclamate studies showed that the sweetener could produce cancer in animals and, as a result, this substance was removed from a wide variety of products. Similar studies have been carried out on saccharin.

Cyclamates and saccharin have been banned in some countries as ingredients in manufactured products. However, these substances may still be purchased as OTC products themselves. Much research has been done to find a safe synthetic substitute for sucrose. As a result aspartame (methyl *N*-L-α-aspartyl-L-phenylalaninate), which is about 200 times sweeter than sucrose, is now being used in many commercial preparations as the sweetening agent. It is sparingly soluble in water and is most stable at a pH of 4.3. This compound will likely be used in a number of pharmaceutical formulations in the near future.[14]

Incompatibilities—Since elixirs contain alcohol, incompatibilities of this solvent are an important consideration during the formulation phase. Alcohol precipitates tragacanth, acacia, and agar from aqueous solutions. Similarly, it will precipitate many inorganic salts from similar solutions. The implication here is that such substances should be absent from the aqueous phase or should be present in such concentrations that there is no danger of precipitation on standing.

If an aqueous solution is added to an elixir, a partial precipitation of ingredients may occur. This is due to the reduced alcohol content of the final preparation. Usually, however, the alcohol content of the mixture is not sufficiently high to cause separation. As vehicles for tinctures and fluidextracts, the elixirs generally cause a separation of extractive matter from these products due to a reduction of the alcohol content.

Many of the incompatibilities between elixirs and the substances combined with them are due to the chemical characteristics of the elixir *per se* or of the ingredients in the final preparation. Thus certain elixirs are acid in reaction while others may be alkaline and will, therefore, behave accordingly.

Glycerins

Glycerins or glycerites are solutions or mixtures of medicinal substances in not less than 50% by weight of glycerin. Most of the glycerins are extremely viscous and some of them are of a jelly-like consistency. Few of the glycerins are extensively used.

Glycerin is a valuable pharmaceutical solvent forming permanent and concentrated solutions not otherwise obtainable. Some of these solutions are used in their original form as medicinal agents while others are used to prepare aqueous and alcoholic dilutions of substances which are not readily soluble in water or alcohol. Glycerin Otic Solution of the USP is discussed previously under Otic solutions. One of the glycerins, Phenol Glycerin BPC is diluted with glycerin to form the pharmaceutical preparation, Phenol Ear-Drops BPC.

Phenol Glycerin BPC

Phenol	160 g
Glycerin	840 g

Dissolve the phenol in the glycerin.

Phenol Ear-Drops BPC

Phenol Glycerin	40 mL
Glycerin, a sufficient quantity, to make	100 mL

Add the glycerin to the glycerite.

Water must not be added to this preparation. It reacts with the phenol to produce a preparation which is caustic and, consequently, damaging to the area of application.

Although not within the context of the definitions given in this section, certain aqueous and nonaqueous preparations are used to remove wax (cerumen) from the ear. One commercially available preparation contains benzocaine, chlorbutol, *p*-dichlorobenzene, and turpentine; others contain olive oil, dioctyl sodium sulfosuccinate, or triethanolamine polypeptide oleate-condensate. Sodium Bicarbonate Ear-Drops BPC should be used if wax is to be removed from the ear. This preparation contains sodium bicarbonate (5 g), glycerin (30 mL), and purified water (a sufficient quantity to make 100 mL).

Starch Glycerin, an emollient, contains starch (100 g), benzoic acid (2 g), purified water (200 mL), and glycerin (700 mL).

Glycerins are hygroscopic and should be stored in tightly closed containers.

Inhalations and Inhalants

Inhalations

These preparations are so used or designed that the drug is carried into the respiratory tree of the patient. The vapor or mist reaches the affected area and gives prompt relief from the symptoms of bronchial and nasal congestion. The USP defines Inhalations in the following way:

> Inhalations are drugs or solutions of drugs administered by the nasal or oral respiratory route for local or systemic effect. Examples in this Pharmacopeia are Epinephrine Inhalation and Isoproterenol Hydrochloride Inhalation. Nebulizers are suitable for the administration of inhalation solutions only if they give droplets sufficiently fine and uniform in size so that the mist reaches the bronchioles.
>
> Another group of products, also known as inhalations and sometimes called insufflations, consists of finely powdered or liquid drugs that are carried into the respiratory passages by the use of special delivery systems such as pharmaceutical aerosols that hold a solution or suspension of the drug in a liquefied gas propellant (see Aerosols). When released through a suitable valve and oral adapter, a metered dose of the inhalation is propelled into the respiratory tract of the patient. Powders may also be administered by mechanical devices that require a manually produced pressure or a deep inspiration by the patient, eg, *Cromolyn Sodium*.
>
> Solutions may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizer, or the nebulizer may be attached to a plastic face mask, tent, or intermittent positive-pressure breathing (IPPB) machine.

As stated in the pharmacopeia, particle size is of major importance in the administration of this type of preparation. The various types of mechanical devices that are used in conjunction with inhalations are described in some detail in Chapter 104. It has been reported in the literature that the optimum particle size for penetration into the pulmonary cavity is of the order of ½ to 7 μm. Fine mists are produced by pressurized aerosols and hence possess basic advantages over the older nebulizers. In addition to this, metered aerosols deliver more uniform doses than those obtained with the older mechanical devices. Chapter 93 should be consulted for further details on this subject.

The term *Inhalation* is used commonly by the layman to represent preparations intended to be vaporized with the aid of heat, usually steam, and inhaled. Benzoin Inhalation BPC contains benzoin, storax, and alcohol. The vapors from a preparation containing 1 teaspoonful of the tincture and 1 qt of boiling water may be inhaled. The device known as a *vaporizer* is used with a number of commercially available preparations of this type.

Epinephrine Inhalation and Isoproterenol Hydrochloride Inhalation are described in USP.

Chapter 24 / Asthma

H. William Kelly, PharmD, and Malcolm R. Hill, PharmD

Bronchial asthma is a common disease of children and adults. Although the clinical manifestations of asthma have been known since antiquity, it is a disease that still defies precise definition. The word *asthma* is of Greek origin and means panting. More than 2000 years ago, Hippocrates used the word asthma to describe episodic shortness of breath; however, the first detailed clinical description of the asthmatic patient was made by Aretaeus in the second century.[1] Since that time asthma has been used to describe any disorder with episodic shortness of breath or dyspnea; thus, the terms *cardiac asthma* and *bronchial asthma* have been used to delineate the etiologies of the dyspnea. These terms are now obsolete and asthma refers to a disorder of the respiratory system characterized by episodes of difficulty in breathing. The Committee on Diagnostic Standards of the American Thoracic Society[2] defined asthma as "a disease characterized by an increased responsiveness of the trachea and bronchi to a variety of stimuli and manifested by widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy." A committee of the American Thoracic Society has defined reversibility as "a significant improvement in measurement(s) of airway obstruction greater than 1.65 times the coefficient of variation of the test(s) used to assess reversibility of the airways."[3] Airway obstruction was characterized as "presence of a significant obstructive ventilatory abnormality in tests of ventilatory mechanics" and "presence of associated periodic cough, tightness, wheezing, or dyspnea."

The lack of a more precise definition for asthma is attributed to our lack of knowledge of the precise pathogenic defect that results in the clinical syndrome we recognize as asthma. Thus, the definition represents a description of the clinical symptoms of asthma without a delineation of etiology. The current definition does allow for the important heterogeneity of the clinical presentation of asthma. New technologies have added substantially to our understanding of the interrelationships of immunology, biochemistry, and physiology to the clinical presentation of asthma, and further research may yet uncover a single cellular defect in asthma. Until such time, asthma will continue to defy exact definition.

Epidemiology

The lack of a precise uniform definition of asthma has hampered epidemiologic research in asthma. Depending on the definition, between 7 and 20 million people in the United States (about 5% of the population) have asthma.[4] Children make up 2 to 5 million of the total. The estimated economic cost of asthma in the United States in 1985 was $4 billion.[4] Childhood asthma is a major cause of school absenteeism; children with asthma have absentee rates 24% higher than overall absentee rates. Two population-based studies reported that 50% of all subjects had an age of onset younger than 10 years and that asthma below the age of 15 occurs primarily in boys while older asthmatics are more commonly girls and women.[5] The prevalence of asthma in the United States has increased 33% between 1970 and 1987 from 30.2 to 40.1 per 1000 with blacks having a 19% higher incidence than whites.[4]

Natural History

Weiss and Speizer[5] recently reviewed the long-têrm follow-up studies of patients with asthma published since 1952. They noted that a number of the studies had serious methodologic problems. Many of the studies were retrospective and either clinic or hospital based, leading to possible bias in patient selection. In these studies, the definition of asthma was often unclear and physiologic tests of airway reactivity were not performed. Population-based controls were used in only one study. Despite these limitations, some conclusions about the natural history of asthma can be made. Between 30% and 70% of children with asthma will markedly improve or become symptom-free by early adulthood; chronic disease persists in about 30% of patients. Although asthmatic patients who develop the disease in childhood are more likely to have remissions, patients who present at an early age have a poorer prognosis.

Approximately 60% of patients who are symptom-free as adults continue to exhibit bronchial hyperreactivity to inhaled histamine challenges. The Tucson Epidemiologic Study reported a 28.7% remission rate that was highest in the 10- to 19-year-old age group (65%) and lowest in the 40- to 49-year-old subjects (6%).[6] Subjects with less frequent attacks and normal pulmonary function on initial assessment had higher remission rates. In addition, smokers had the lowest remission and highest relapse rates.

Although death from asthma is still relatively uncommon, there has been an increasing national concern over asthma mortality. This is because the death rate from asthma has doubled from 2600 to 4000 per year between 1979 and 1987. This is consistent with the increase in death rates found in Canada, Australia, West Germany, England, and Wales.[7] The greatest increase in asthma deaths has occurred in those older than 65 years of age. Blacks have nearly twice the death rate from asthma as whites. This discrepancy is even more evident in children where the death rate for blacks was

three to nine times higher than for whites in children 10 to 14 years old.[4]

Studies of the cause and prevention of death from asthma have presented disturbing results. They indicate that 80% to 90% of the deaths are preventable.[7] Most deaths from asthma occur outside of the hospital and death is rare after hospitalization.[7] The most common cause of death from asthma is inadequate assessment of the severity of airway obstruction by the patient or physician and inadequate therapy.[7] This was found to be true in deaths of hospitalized patients as well as outpatients; thus the key to prevention of death from asthma as advocated by the Division of Lung Diseases of the National Heart Lung and Blood Institute and others is education.[4] This includes education of the patients as well as the clinicians caring for them.

Pathophysiology

Bronchial Hyperreactivity

Although a single underlying cellular defect has not yet been discovered, new technologies have substantially advanced our understanding of the pathogenesis of asthma. Hyperreactivity of the airways to physical, chemical, and pharmacologic stimuli is the hallmark of asthma.[8] Bronchial hyperreactivity also occurs in some patients with chronic bronchitis and allergic rhinitis, though to a lesser degree.[8] Normal healthy subjects may also develop a transient increased bronchial reactivity after viral respiratory infections or exposure to ozone.[8] However, the degree of reactivity is quantitatively greater in asthmatic patients than in other groups who demonstrate hyperreactivity. Bronchial reactivity of the general population fits a unimodal distribution that is skewed toward increased reactivity.[5] Patients with clinical asthma represent the extreme end of the distribution. The degree of bronchial hyperreactivity with asthmatics correlates with the clinical course of their disease and medication requirement necessary to control symptoms.[8] Patients with mild symptomology or in remission demonstrate lower levels of reactivity, though still greater than the normal population. Much of the recent research on the pathogenesis of asthma has focused on explaining airway hyperreactivity. A number of excellent reviews and symposia have highlighted new discoveries and summarized the current state of knowledge of this expanding area of research.[8,9]

Our current understanding recognizes that the increased bronchial responsiveness seen in asthma is at least in part due to an inflammatory response within the airway.[10] The intact lungs of patients at autopsy are hyperinflated because of air trapping from widespread mucus plugging. The histologic examination is characterized by three findings: (1) marked hypertrophy and hyperplasia of the airway smooth muscle, (2) increased airway wall thickness caused by an exudative inflammatory reaction and edema, and (3) mucous gland hypertrophy and mucus hypersecretion[8] (Fig. 24.1). Although the precise link is not known, bronchial hyperresponsiveness is thought to be related to the extent of inflammation in the airways.

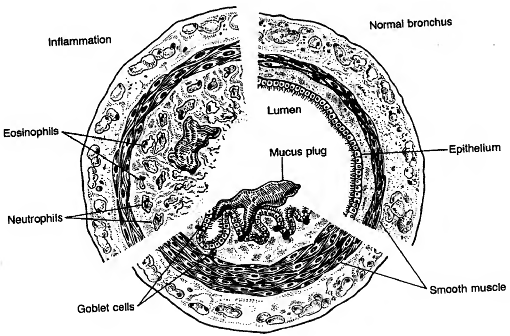


Figure 24.1 Representative illustration of the pathology found in the asthmatic bronchus compared with a normal bronchus (upper right). Each section demonstrates how the lumen is narrowed. Edema of the basement membrane, mucus plugging, smooth muscle hypertrophy, and constriction contribute (lower section). Inflammatory cells producing epithelial desquammation fill the airway lumen with cellular debris and expose the airway smooth muscle to other mediators (upper left).

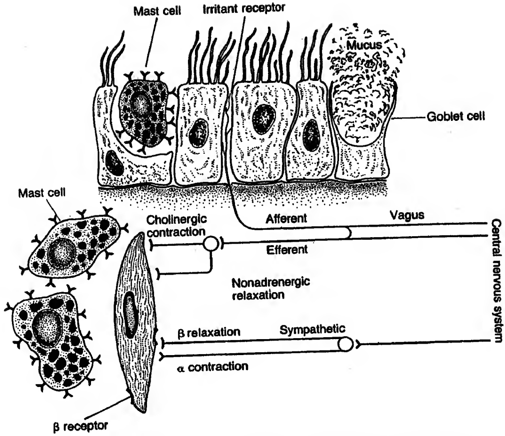


Figure 24.2 Innervation of the airways by the sympathetic, cholinergic, and nonadrenergic inhibitory systems. Mast cell concentration increases from the epithelial lumen to the submucosa.

Histologic Changes in the Lining of the Airways

Histologic studies performed on patients with mild-intermittent to moderate-chronic asthma have shown marked inflammatory changes within the airway along with extensive epithelial damage.[10] Similar but more severe changes have also been seen in patients who have died from acute asthma attacks.[8,9] The correlation between the degree of epithelial denudation and airways reactivity suggests that patients with the most reactive airways have the least amount of normal bronchial epithelium.[11] Subepithelial fibrosis has also been described in the bronchi of patients with mild asthma.[12] This finding is not surprising considering that fibrosis occurs as a result of other chronic inflammatory diseases, such as colitis.[13] It is not known whether the epithelial damage precedes the development of reactive airways or if these features occur simultaneously. It is also not known whether epithelial damage is a universal feature in all patients. The presence of subepithelial fibrosis is of most concern as it remains to be determined whether this type of fibrosis represents a reversible or transient process, or can have more serious sequelae such as the development of chronic obstructive pulmonary disease.

Cellular and Biochemical Features

Inflammatory Processes

The inflammatory reaction appears to be the key mechanism to explain the pathologic changes seen in asthma. In addition, inflammation of the airways and the release of mediators of inflammation appears to be necessary for the development and maintenance of bronchial hyperreactivity.[8,10] Inflammation of the airways is associated with epithelial cell damage and increased mucosal permeability.[8] This improves access of noxious stimuli from the lumen to the airway smooth muscle, submucosal mast cells, and the cholinergic irritant receptors located in the junction between cells[8,10] (Fig. 24.2). The epithelial damage and turnover leads to the hypertrophy of the basement membrane.[8] Inflammation can also account for mucus hypersecretion.[8] Therefore, recent research on the pathogenesis of bronchial hyperreactivity has focused on inflammation and the mediators of the inflammatory process. Figure 24.3 lists a number of identified mediators, their origin, and pathophysiologic processes with which they are associated.[8,10] While our understanding of the complex interactions involved in the inflammatory process is still incomplete, the central role of inflammation in

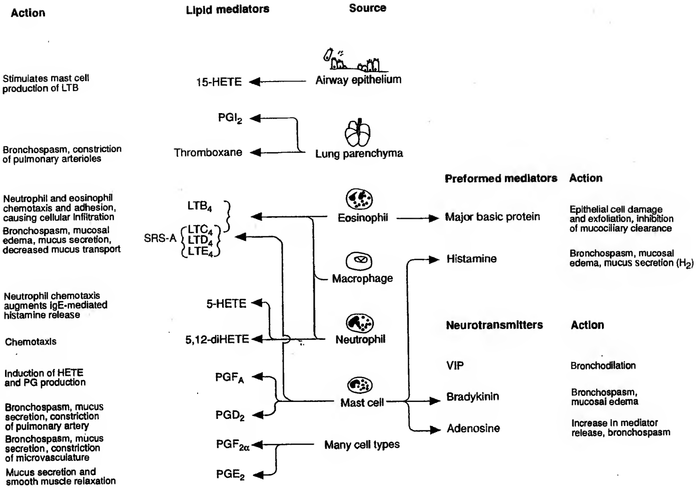


Figure 24.3 Diagrammatic presentation of the source, variety, and pathogenetic effects of lipid and preformed mediators as well as neurotransmitters involved in asthma. See text for details. HETE, hydroxyeicosatetaraenoic acid; PG, prostaglandin; LT, leukotriene; SRS-A, slow-reacting substance of anaphylaxis; VIP, vasoactive intestinal polypeptide.

producing or increasing bronchial hyperreactivity appears clear. With the increased awareness of the role of inflammation in the pathogenesis of asthma, it becomes apparent that therapy directed solely at bronchospasm is incomplete. Attempting to minimize inflammation is an important aspect of asthma therapy.

Inflammatory Cells

Numerous types of leukocytes are present in the circulation, lung tissues, and lumen of airways. The involvement of mast cells, eosinophils, neutrophils, alveolar macrophages, and lymphocytes within the airways and surrounding tissues are important in asthma. The contributions of these cell types to asthma are important in terms of pathogenesis and for understanding how therapy may alter cellular presence and function. Future therapeutic strategies aimed specifically at decreasing the number of inflammatory cells or removing their effect on asthma pathogenesis are being developed.

Mast Cells The mast cell is thought to be important in the initiation of immediate responses following exposure to allergens. Mast cells are found throughout the walls of the respiratory tract and increased numbers of these cells (threefold to fivefold) have been described in the airways of asthmatics with an allergic component.[8] Once binding of allergen to cell-bound immunoglobulin E (IgE) occurs, mediators such as histamine; eosinophil and neutrophil chemotatic factors; leukotrienes C_4, D_4, and E_4; prostaglandins; platelet-activating factor; and others are released from mast cells (Fig. 24.3). Upon histologic examination, decreased numbers of granulated mast cells have been observed in the airways of patients who have died from acute asthma attacks, suggesting that mast cell degranulation is a contributing factor in the progression of the disease.[14]

Eosinophils Eosinophils have long been linked to asthma, primarily due to the association between asthma and peripheral blood eosinophilia.[15] The degree of bronchial reactivity has been related to the number of eosinophils in peripheral blood and bronchoalveolar lavage fluid.[15] Major basic protein (MBP), a constituent of the granules present in the eosinophil, is responsible for damage to airway epithelium

and has been found in very high quantities in the sputum of patients with asthma.[15] The damage to airway epithelium caused by eosinophil degranulation and the subsequent disruption of normal physiology may contribute to development of the airway hyperreactivity characteristic of asthma.[15]

Alveolar Macrophages The role of alveolar macrophages in the pathogenesis of asthma is becoming better understood.[10] The primary function of these cells in the normal airway is to serve as "scavengers," engulfing and digesting bacteria and other foreign materials. They are found in large and small airways, ideally located in terms of their potential for affecting the asthmatic response. A number of mediators produced and released by macrophages have been identified and their roles in initiating and amplifying inflammation in allergic asthma have been determined.[16] A partial list of mediators produced by these cells includes platelet-activating factor, leukotriene B_4, leukotriene C_4, and leukotriene D_4. Additionally, alveolar macrophages are able to produce neutrophil chemotactic factor and eosinophil chemotactic factor.[17] These substances attract neutrophils and eosinophils, which in turn further facilitate the inflammatory process.

Lymphocytes A study in guinea pigs revealed an accumulation of T lymphocytes in bronchial mucosa, corresponding to allergen-induced late asthmatic response.[17] In humans, it has been shown that increased numbers of CD4+ cells are found in the biopsies of cutaneous late-phase responses after dermal allergen challenge.[8] Another study revealed that T lymphocytes and their activation markers in peripheral blood were increased in acute severe asthma, and decreased with treatment and clinical improvement.[8] These findings along with the potential capability of T lymphocytes to up-regulate the immune response suggest that the role of these cells in asthma may be more important than previously believed. The recent description of the efficacy of methotrexate in severe asthma may be related to the effect of this drug on proliferation and immunomodulation of the T lymphocyte.

Preformed Mediators

Associated with asthma for many years, histamine is capable of inducing smooth muscle constriction and bronchospasm and is thought to play a role in mucosal edema and mucus secretion.[14] Lung mast cells are an important source of histamine. The release of histamine can be stimulated by exposure of the airway to a variety of factors including physical stimuli (such as exercise) and exposure to relevant allergen.[14]

Membrane-Derived Lipid Mediators

Chemical substances known as phospholipids are found in rich supply in the membranes of most cells involved with inflammation. Several classes of important mediators, including arachadonic acid and its metabolites, prostaglandins, leukotrienes, and platelet-activating factor, are derived from these membrane phospholipids (Fig. 24.3).

Prostaglandins Once arachadonic acid is released it can be broken down by the enzyme cyclooxygenase to form the prostaglandins. A further breakdown product, prostaglandin D_2, has been well characterized and is a potent bronchoconstricting agent.[10] It is unlikely that prostaglandin D_2 can produce sustained effects on airway function or inflammation; however, its role in asthma remains to be determined. Similarly, prostaglandin $F_{2\alpha}$ is a potent bronchoconstrictor in patients with asthma, and can enhance the effects of histamine.[8] It is not clear whether this substance has any other pathophysiologic effects and its specific origin from within the lung is also unknown. Another cyclooxygenase product, prostacyclin (prostaglandin I_2) is known to be produced in the lung. It is unclear whether prostaglandin I_2 is important as a bronchoconstricting agent in humans; however, it may contribute to inflammation and edema due to its effects as a vasodilator.[8]

Thromboxanes The cyclooxygenase products known as thromboxanes have received increasing attention. Of these, thromboxane A_2 is the most understood. Thromboxane A_2 is produced by alveolar macrophages, fibroblasts, epithelial cells, neutrophils, and platelets within the lung.[10] Indirect evidence from animal models suggests that thromboxane A_2 may have several properties, including bronchoconstriction, involvement in the late asthmatic response, and involvement in the development of airways inflammation and hyperreactivity. Potent and specific thromboxane synthetase inhibitors will be crucial tools for understanding the role of thromboxanes in asthma.

Leukotrienes The lipoxygenase pathway of arachadonic acid breakdown is responsible for production of the class of compounds called leukotrienes. Leukotrienes C_4, D_4, and E_4 (sulfidopeptide leukotrienes) constitute the slow-reacting substance of anaphylaxis (SRS-A).[10] These leukotrienes are liberated during inflammatory processes in the lung and have significant effects on airway smooth muscle (bronchoconstriction), mucociliary function, microvascular permeability, and airways edema.[10] In theory, potent leukotriene antagonists should be able to prevent or reverse some of the pathogenetic features of asthma.

Platelet-Activating Factor Thought to be produced by macrophages, eosinophils, and neutrophils within the lung, platelet-activating factor (PAF) is involved in the mediation of many of the important steps in the development of the asthmatic response. These steps include immediate bronchoconstriction and sustained induction of airway hyperreactivity, edema formation, and cellular changes associated with generalized inflammatory responses, including chemotaxis of eosinophils.[10] PAF is the only mediator known to produce a sustained increase in bronchial reactivity.[10] As selective and potent PAF-receptor antagonists are developed and clinical trials are completed, the relative importance of PAF as a mediator in asthma will be more completely understood.

Mucus Production

The mucociliary system is the lung's primary defense mechanism against irritants and infectious agents. Mucus is composed of 95% water and 5% glycoproteins and is produced by bronchial epithelial glands and goblet cells.[9] The lining of the airway consists of a continuous aqueous layer controlled

by active ion transport across the epithelium where water moves toward the lumen along the concentration gradient. Catecholamines and vagal stimulation enhance the ion transport and fluid movement.[9] Mucus transport is dependent on the viscoelastic properties of the mucus. Mucus that is either too watery or too viscus will not be optimally transported. The exudative inflammatory process and sloughing of epithelial cells into the airway lumen adversely affect the mucociliary transport. The bronchial glands are increased in size and the goblet cells are increased in size and number in asthma, suggesting an increased production of mucus.[9] Expectorated mucus from patients with asthma tends to have a high viscosity. The mucus plugs in the airways of patients who died in status asthmaticus are tenacious and tend to be connected by mucus strands to the goblet cells.[9] Asthmatic airways may also become plugged with casts consisting of epithelial and inflammatory cells. While it is tempting to speculate that death from asthma attacks is a result of the mucus plugging resulting in irreversible obstruction, there is no direct evidence for this. Autopsies of asthmatics who died from other causes have shown similar pathology.[7] In addition, some subjects who have died of sudden severe asthma did not show the characteristic mucus plugging on necropsy.[7,9]

Airways Smooth Muscle

The smooth muscle of the airways does not form a uniform coat around the airways but is wrapped around in a connecting network best described as a spiral arrangement.[8,9] The muscle contraction displays a sphincteric action that is capable of completely occluding the airway lumen. The airway smooth muscle extends from the trachea through the respiratory bronchioles. When expressed as percent of wall thickness, the smooth muscle represents 5% of the large central airways and up to 20% of the wall thickness in the bronchioles.[8] Total smooth muscle mass decreases rapidly past the terminal bronchioles to the alveoli so the contribution of smooth muscle tone to airway diameter in this region is relatively small.[8] In the large airways of asthmatics, smooth muscle may account for 11% of the wall thickness.[8] Airway smooth muscle contraction in vivo is measured indirectly by determining the flow of air into and out of the patient. The difficulties in using changes in airflow as a measurement of smooth muscle contraction have been delineated elsewhere.[8] The relationship between airway diameter and flow is dictated by Poiseuille's law.[18]

$$\Delta P = \frac{8nl}{\pi r^4}$$

where n = viscosity of the air, l = length of the tube, r = radius of the tube, and ΔP = drop in pressure. As resistance is equal to P divided by airflow, a twofold change in airway diameter would produce a 16-fold change in airflow resistance. It is possible that the increased smooth muscle mass of the asthmatic airways is important in magnifying and maintaining bronchial hyperreactivity in chronic asthma.[8,9] However, it appears that the hypertrophy and hyperplasia are secondary processes caused by chronic stimulation and are not the primary cause of bronchial hyperreactivity.[8,9]

Nervous System

The airway is innervated by parasympathetic, sympathetic, and nonadrenergic inhibitory nerves (Fig. 24.2). Parasympathetic innervation of the smooth muscle consists of efferent motor fibers contained in the vagus nerves and sensory afferent fibers in the vagus and other nerves.[8] The normal resting tone of human airway smooth muscle is maintained by vagal efferent activity.[8] Maximum bronchoconstriction mediated by vagal stimulation occurs in the small bronchi and is absent in the small bronchioles.[8,10] The nonmyelinated C fibers of the afferent system lie immediately beneath the tight junctions between epithelial cells lining the airway lumen.[8] These endings probably represent the irritant receptors of the airways. Stimulation of these irritant receptors by mechanical stimulation, chemical and particulate irritants, and pharmacologic agents such as histamine produces reflex bronchoconstriction.[8,10] The sympathetic innervation of the airway smooth muscle is sparse and does not directly control airway smooth muscle tone.[10] All airway smooth muscle contains noninnervated β_2-adrenergic receptors that produce bronchodilation.[10] Circulating catecholamines play an important role in regulating bronchial tone. There is some evidence for α-adrenergic receptors in the major resistant airways. Stimulation of these receptors produces bronchoconstriction that is enhanced by pretreatment with histamine.[10] The importance of these receptors in asthma is unknown; however, specific α-adrenergic blockers have minimal effect on asthma.[10] One theory on the pathogenesis of bronchial hyperreactivity is that asthma represents a relative β-adrenergic blockade. The demonstration of a β-adrenergic defect in asthmatic patients has been inconsistent, and the production of β-blockade in normal subjects is insufficient, by itself, to cause bronchial hyperreactivity. Recent studies have suggested the existence of a nonadrenergic, noncholinergic (NANC) nervous system in the trachea and bronchi. The importance of this system is still unknown largely because the exact neurotransmitters are unknown. It has recently been postulated that a defect in the nonadrenergic inhibitory system exists in asthma, as a result of the lack of significant amounts of the neurotransmitter vasoactive intestinal peptide (VIP).[10] NANC excitatory neuropeptides such as substance P and neurokinin A are released by C-fiber sensory nerve endings.[10] The NANC system may play an important role in amplifying inflammation in asthma.

Clinical Presentation

The heterogeneity of asthma appears most obvious when listing the diverse triggers of bronchospasm in asthmatic subjects (Table 24.1). In the past, a good deal of the confusion concerning the definition and etiology of asthma centered on the inclusion of the various triggering events as the etiology. Thus, asthma has been variously defined as an allergic, emotional, and infectious disease. However, it has become clear that asthma is first a lung disease, and specific triggering events have relative degrees of importance from patient to patient. Epidemiologic studies support the concept of a genetic predisposition to the development of asthma.[5] Studies of occupational asthma and the induction of hyperreactivity in healthy individuals emphasize the effect of environment on the development of asthma.[5,8] Asthma is

Table 24.1 Representative List of Agents and Events Triggering Asthma

Trigger	*Mechanism*
Respiratory infection Respiratory syncytial virus (RSV), rhinovirus, influenza, and parainfluenza, *Mycoplasma* pneumonia	Inflammation and epithelial damage sensitizing cholinergic irritant receptors; virus-induced relative β-blockade possibly contributes
Allergens Airborne pollens (grasses, trees, weeds), house dust, animal danders, dust mites, insect parts, fungal spores, foods	IgE-mediated mast cell mediator release
Exercise	Hyperventilation with loss of water and cooling of the airways and mast cell mediator release
Occupational stimuli Animal handlers; antibiotic drug manufacturing; bakers (flour dust); woodworkers; spice and enzyme workers; plastic workers (anhydrides); printers (arabic gum); chemical workers (azo dyes, anthraquinone, ethylenediamine, toluene diisocyanates, meat wrappers (heated polyvinyl chloride); plastics, rubber, and wood workers (formaldehyde, western red cedar, dimethylethanolamine)	IgE-mediated mast cell release Airway epithelial damage, increased permeability and sensitization of irritant receptors
Environment Cold air, ozone, sulfur dioxide, nitrogen dioxide	Unknown (irritation?); epithelial damage and neutrophil infiltration
Emotions Anxiety, fatigue, stress, laughter	Parasympathetic stimulation; augments preexisting event, generally not a primary event
Drugs	See text for discussion

still frequently classified according to its predominant trigger; however, it should be emphasized that this method of classification is at best arbitrary and many patients respond to a number of stimuli. Indeed it is the uniform increased responsiveness to challenge with the nonspecific stimuli methacholine, histamine, and exercise that is often used to define and diagnose asthma.

Chronic Asthma

Classic asthma is characterized by episodic dyspnea associated with wheezing; however, the clinical presentation of asthma is as diverse as the number of triggering events. Although wheezing is the characteristic symptom of asthma, the medical literature is replete with the warning that "not all that wheezes is asthma." A wheeze is a high-pitched, whistling sound created by turbulent airflow through an obstructed airway so that any condition that produces significant obstruction can result in wheezing as a symptom. In addition, "all of asthma does not wheeze" is an equally justifiable warning. Patients may present with a chronic persistent cough as their only symptom.[10]

The diagnosis of asthma is based primarily on a good history of recurrent episodes of dyspnea and/or wheezing. The patient may complain of a feeling of tightness in the chest or sometimes a burning sensation. The patient may have a family history of allergy or asthma or have symptoms of allergic rhinitis. A history of exercise or cold air precipitating the dyspnea or an association of increased symptoms during specific allergen seasons would also point to asthma.

Asthma has a widely variable presentation from chronic daily symptomology to only intermittent symptoms. The intervals between symptoms could be weeks, months, or years. It is a disease characterized by recurrent exacerbations and remissions. The next variable is severity. The intermittent and/or chronic nature of symptoms does not necessarily determine the severity of symptoms during exacerbations. The severity is primarily determined by the number of medications required to adequately control the patients' symptoms. Patients can present with mild intermittent symptoms that require no medications or only occasional use of inhaled bronchodilators to severe chronic asthmaticus.

Chronic Severe Asthma

Chronic severe asthma is defined by the requirement of continuous or frequent intermittent glucocorticoids for control of symptoms. These patients frequently demonstrate significant residual pulmonary function abnormalities and require chronic bronchodilator therapy for control of symptoms. These patients can be frustrating for the general practitioner and specialist alike, and therefore it is fortunate, that they comprise a relatively small percentage of all asthmatics. Short lapses in compliance with the treatment regimen can lead to hospitalization with life-threatening asthma attacks. The most severe patients may infrequently require hospitalization for acute attacks of asthma despite compliance with maximum dosages of chronic medications. In this sense, they are not unlike the "brittle diabetics" who

need only a small insult to tip the balance. These patients are in the greatest danger of death from asthma and present the greatest challenge to the clinicians caring for them. The clinician is frequently forced to assess the risk:benefit ratio of the therapeutic plan because it is frequently necessary to use a number of drugs including daily long-term oral glucocorticoids at their maximum recommended dosages.

Acute Severe Asthma

Uncontrolled asthma, with its inherent variability, can progress to an acute state where inflammation, airways edema, excessive accumulation of mucus, and severe bronchospasm result in a profound airways narrowing which is poorly responsive to usual bronchodilator therapy. This clinical situation is a common circumstance necessitating emergency room admission. In many cases, emergency room visits for acute severe asthma represent the failure of an adequate therapeutic regimen for chronic asthma. Patients present with severe dyspnea, inspiratory as well as expiratory wheezing, anxiety, tachypnea, tachycardia, and in severe cases cyanosis. They exhibit supraclavicular and intercostal retractions, a hyperinflated chest, and coughing. In severe obstruction, air movement in and out of the lungs is substantially decreased so that wheezing may actually decrease.

Etiology

Allergic Asthma

An allergic component can be demonstrated in 35% to 55% of asthmatic patients, and this may be higher in childhood asthma.[5] The allergens (Table 24.1) that provoke asthma are airborne and evoke the asthmatic response through the classical allergic pathway, depicted in Fig. 24.4. The role of allergy in the etiology of asthma has been controversial and asthma has been considered an allergic disease by many. Although the allergic reaction plays an important role in the atopic asthmatic patient, atopy is not necessary for the development of asthma and not all atopic individuals develop asthma. Many patients with hay fever will develop some airway hyperreactivity (though less than asthmatics) during their allergen season.

When allergic asthmatics are given an inhalational challenge with an allergen to which they are sensitized, the patients demonstrate an immediate asthmatic reaction (Fig. 24.5). The reaction is characterized by a drop in pulmonary function that reaches maximum intensity in 10 to 20 minutes and reverses spontaneously by 60 to 120 minutes.[8] In addition, many subjects experience a late asthmatic reaction (Fig. 24.5) that begins 4 hours after the challenge, reaches maximum intensity in 6 to 8 hours, is often more severe than the immediate response, and may last as long as 24 hours. The late asthmatic reaction (LAR) has engendered renewed interest from many researchers who believe that it may be the pathogenetic mechanism for inducing and maintaining bronchial hyperreactivity in atopic asthmatics.[8] Patients who experience an LAR demonstrate increased responsiveness to methacholine, histamine, and exercise that may last up to 6 weeks, while subjects who only experience the immediate response demonstrate no increased bronchial reactivity.[8] The degree of hyperresponsiveness and its duration correlate with the intensity of the LAR. The LAR is associated with increased serum concentrations of neutrophil and eosinophil chemotactic factors and the influx of neutrophils and eosinophils into the tissue as well as the degranulation of mast cells.[8] The LAR is associated with greater degrees of obstruction in small airways and air trapping than occur in the immediate response. The immediate response, due to the degranulation of mast cells and histamine release, is easily blocked or reversed with inhaled β_2-adrenergic agents. Theophylline, anticholinergics, and oral β_2-agonists blunt the response but are inconsistently effective. The LAR is not prevented by pretreatment with any of these bronchodilators and often does not respond as well to them as does the immediate response. Glucocorticoid pretreatment does not alter the early response but prevents the LAR, whereas pretreatment with cromolyn sodium blocks both responses. Long-term treatment with glucocorticoids can attenuate the immediate response.

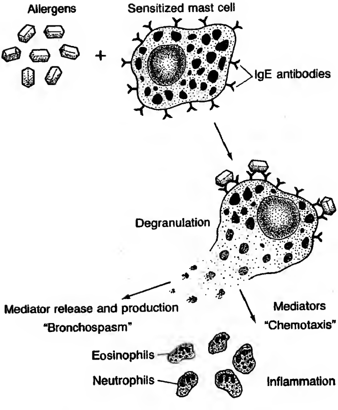


Figure 24.4 IgE-mediated allergic response. Allergens produce steric changes in a mast cell, leading to the spillage of granules with preformed mediators as well as initiating production of other mediators of anaphylaxis through the lipoxygenase pathway.

Clinically, allergic asthmatics develop increased bronchial hyperreactivity with increased exposure to allergens during a pollen season. Avoidance of the pollen or prophylaxis with cromolyn sodium prevents the increased bronchial hyperreactivity.[8] Studies have shown that long-term therapy with both cromolyn sodium and glucocorticoids reduces bron-

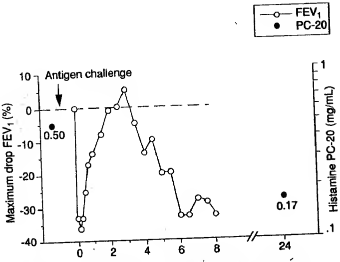


Figure 24.5 Biphasic response to allergen exposure in a sensitive patient with asthma. The immediate response occurs within 10 to 30 minutes following exposure, and may revert to baseline without intervention. The late asthmatic response occurs within 2 to 8 hours following exposure. The provocative concentration of inhaled histamine, which produces a 20% decrease in forced expiratory volume in 1 second (FEV_1) (PC–20), an index of airways reactivity, also shows a marked decrease following development of a late asthmatic response. This is suggestive of an increase in the propensity of the airways to constrict to nonspecific stimuli.

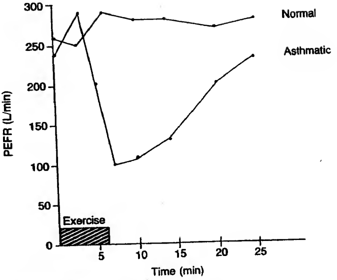


Figure 24.6 Typical responses to exercise in a normal subject and an asthmatic subject. Note the initial bronchodilation. PEFR, peak expiratory flow rate.

chial hyperreactivity.[8] In contrast, long-term therapy with β_2-adrenergics and methylxanthines has not been associated with similar decreases in bronchial hyperreactivity.[8]

Exercise-Induced Asthma

During vigorous exercise, pulmonary function in asthmatic patients (as measured by forced expiratory maneuvers) increases during the first few minutes but then begins to decrease after 6 to 8 minutes (Fig. 24.6). Exercise-induced asthma (EIA) is defined as a drop in forced expiratory volume in 1 second (FEV_1) of greater than 15% to 20% of baseline (preexercise value).[19] Most studies suggest that 70% to 90% of all asthmatics experience EIA.[19] The exact pathogenesis of EIA is unknown; however, heat loss and or water loss from the central airways appear to play an important role.[19] EIA is more easily provoked in cold, dry air, and warm, humid air can blunt or block it.[19]

Studies using isocapnic hyperventilation of cold air have demonstrated similar degrees of bronchospasm as seen in EIA.[8] Whether this is mediated through cooling of the airway or the loss of water is still unknown.[19] A number of studies have demonstrated increased plasma histamine concentrations during EIA, suggesting a role for the mast cell. Recent investigations demonstrating a rise in neutrophil chemotactic factor in asthmatics with EIA but not in healthy individuals or asthmatics without EIA confirms the involvement of mediator release in EIA.[20] In addition, pretreatment with cromolyn sodium, a drug that stabilizes mast cells, inhibits EIA and inhibits the associated rise in neutrophil chemotactic factor.[19] A small number of patients with EIA will have a late response similar to the LAR and associated with a secondary rise in neutrophil chemotactic factor.[20]

A refractory period with EIA lasts up to 3 hours after exercise. During this period, repeat exercise of the same intensity either produces no decrease in pulmonary functions or a drop less than 50% of the initial response.[19] The refractory period is thought to be caused by an acute depletion of mast cell mediators and time required for their repletion.[8] Isocapnic hyperventilation with cold air is not associated with a refractory period.[8] Patients with known refractoriness to exercise will still respond to histamine so that acute hyporesponsiveness of airway smooth muscle does not appear to be a factor.[19]

EIA is believed to be a reflection of the increased hyperreactivity of asthmatics. There is a correlation, though not complete, between EIA and reactivity to histamine and methacholine.[8] Other patient groups with increased airway reactivity (postviral infection, cystic fibrosis, hay fever) show bronchoconstriction after exercise to a lesser degree (5% to 10%) than asthmatics (20% to 40%).[8] Asthmatics will not always demonstrate the same sensitivity. During periods of remission, they often have a decreased sensitivity to the same degree of exercise. However, a number of children and adults with EIA are otherwise normal, without symptoms or abnormal pulmonary function.

Nocturnal Asthma

Worsening of asthma during sleep is referred to as *nocturnal asthma*. Patients with these symptoms have been called "morning dippers" in reference to their significant fall in pulmonary function between bedtime and awakening.[9] Although the pathogenesis of this phenomena is unknown, it has been associated with diurnal patterns of endogenous cortisol secretion and circulating epinephrine.[9] More recent data, however, suggest that this relationship may not be as

clear. Numerous factors that can affect nocturnal worsening of asthma, including allergies and improper environmental control, gastroesophageal reflux, and sinusitis, must also be considered when evaluating these patients.[21]

Miscellaneous Factors

Viral Infections

It has been understood for many years that viral upper respiratory infections are associated with exacerbation of asthma. Well-controlled investigations have convincingly demonstrated that viral infections and not bacteria are primarily responsible for exacerbation of asthma.[5,22] Viral upper respiratory tract infection is a major precipitant of acute asthma in children, and may be important in adults as well. Although the exact mechanism is unknown, the inflammatory response to viral infection is thought to be directly associated with the increasing bronchial hyperresponsiveness.[22] The increase in asthma symptoms and bronchial hyperresponsiveness that occurs may last for days or weeks following resolution of the symptoms of the viral infection.

Environmental and Occupational Factors

Agents and events and the mechanisms that are known to trigger asthma are listed in Table 24.1. The general mechanisms are unknown, but is presumably caused by epithelial damage and inflammation in the airway mucosa. Ozone and sulfur dioxide, common components of air pollution, have been used to induce airways hyperreactivity in animals. Exposure to ozone 0.2 ppm for 2 to 3 hours can induce bronchoconstriction and increase bronchial reactivity in asthmatics.[9] Sulfur dioxide in the ambient atmosphere is highly irritating, but it is not known how it induces bronchoconstriction. Pretreatment with cromolyn sodium will block the obstruction, implicating mast cell or irritant receptor involvement.[9] Asthma produced by repeated prolonged exposure to industrial inhalants is a significant health problem. It has been estimated that occupational asthma accounts for 2% of all asthmatic persons.[9] Persons with occupational asthma have the typical symptoms of asthma with cough, dyspnea, and wheeze. Typically, the symptoms are related to work with improvement on weekends and vacations. In some instances, symptoms may persist even after termination of exposure.[9]

Psychologic Factors

Emotions and stress can rarely precipitate attacks of asthma, but more commonly worsen an attack in progress.[8] Bronchoconstriction from psychologic factors appear to be primarily mediated through excess parasympathetic input. Atropine has been shown to block experimental psychogenic bronchoconstriction.[9] It is most important to emphasize to patients and to parents of asthmatic children that asthma is not an emotional disease; however, calming influences and relaxation techniques may benefit the patient who becomes severely emotionally distraught during asthma attacks.

Sinusitis/Rhinitis

Disorders of the upper respiratory tract, particularly sinusitis and rhinitis, have been linked with asthma for many years. Nasal obstruction or blockage associated with postnasal drip may alter lower airways function and potentially worsen asthma. Transport of mucus chemotactic factors, and inflammatory mediators from nasal passages into the lung may accentuate bronchial hyperresponsiveness. Medical therapy of sinusitis, although empiric, may improve underlying asthma.

Other Factors

Gastroesophageal reflux has been associated with asthma for many years. Reflux of acidic gastric contents into the esophagus is thought to initiate a vagally mediated reflex bronchoconstriction.[21] Also of concern is that most medications that decrease airways smooth muscle tone have a relaxant effect on gastroesophageal sphincter tone as well. The therapeutic approach most commonly taken for patients with gastroesophageal reflux and asthma is to initiate standard antireflux therapy and observe the asthma symptoms.

Blood Gas Measurement

Gas exchange at the alveoli–capillary interface is dependent on ventilation (V_a), or the mechanical properties of the lung, perfusion (Q), the flow of blood, and diffusion of the gases across the membrane. Studies of diffusion capacity in acute asthma indicate that it is slightly increased or unchanged.[9] Arterial hypoxia is common during acute asthma attacks and is caused by significant derangements in V_a/Q relationships.[9] The airway narrowing during asthma attacks though diffuse results in large abnormalities in the distribution of ventilation. The perfusion abnormalities appear to be secondary to changes in ventilation. The normal response to alveolar hypoxia is active vasoconstriction to shunt blood flow to better ventilated areas.[9] Unfortunately, the V_a and Q are not perfectly matched in acute asthma. This may in part be caused by the increased vascular resistance produced by the lung hyperinflation.[9] Figure 24.7 demonstrates the effect of increasing airway obstruction on arterial blood gases. When lungs initially become obstructed, patients demonstrate a marked respiratory drive thought to be caused by the stimulation of the irritant receptors because it is not obliterated by correcting the hypoxemia.[9] As a result, the asthmatic tends to "blow off" carbon dioxide and the arterial carbon dioxide concentration decreases (Fig. 24.7). Unfortunately, the patient is forced to breath at higher lung volumes because of air trapping. This requires the use of accessory respiratory muscles. When the respiratory muscles begin to fatigue, the patient will retain carbon dioxide, and this may signal impending respiratory failure.

Diagnostic Tests

In the older child and adult patient in whom spirometric evaluations can be performed, abnormal pulmonary functions that improve 15% or more following bronchodilator

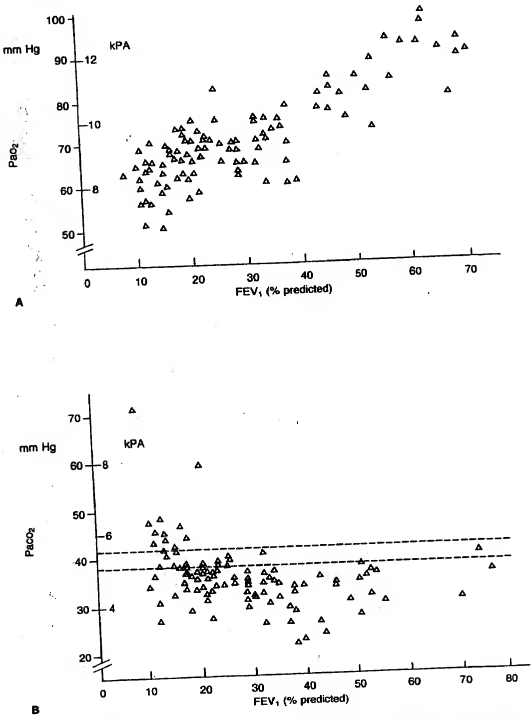


Figure 24.7 Effect of increasing airway obstruction on arterial Pa_{O_2} (A) and Pa_{CO_2} (B). The dashed lines show the normal range of Pa_{CO_2}. *(From McFadden ER Jr, Lyons HA. Arterial blood gas tension in asthma. N Engl J Med 1968;278:1029, with permission.)*

administration helps confirm the diagnosis. Failure of pulmonary functions to improve acutely does not necessarily rule out asthma. Patients with long-standing disease or substantial inflammation may require an intensive prolonged course of bronchodilators and glucocorticoids before reversibility is detected.[9] If baseline spirometry is normal, challenge testing with exercise, histamine, or methacholine can be used to elicit bronchial hyperreactivity. Histamine and methacholine are inhaled in increasing concentrations until the patient's FEV_1 drops at least 20% from the baseline.[8] Standard procedures for performing and interpreting challenge tests have been published.[9] Patients with significant

symptomology should not be challenged due to their increased sensitivity and need not be challenged for diagnostic purposes.[8,9] Spirometry and bronchoprovocation have been shown to be more reliable indicators of bronchial hyperreactivity than history of wheezing and physical exam.[9] Studies for atopy such as serum IgE and eosinophils are not necessary to make the diagnosis of asthma, but they may help differentiate asthma from chronic bronchitis in adults. Clinically, this distinction is often difficult to make. Some patients with chronic bronchitis may have a reversible component and some patients with long-standing severe chronic asthma may have significant irreversible damage and obstruction. Very high peripheral blood eosinophil counts may point to the diagnosis of aspergillosis or other hypereosinophilic syndromes. Skin testing is of no value in diagnosing asthma, but may be useful in identifying etiologic triggers. In small infants unable to perform spirometry, the diagnosis is more difficult. They may demonstrate hyperinflation on the chest roentgenogram. Radiologic exam is helpful in ruling out other causes of wheezing (e.g., foreign body aspiration, parenchymal lung disease, cardiac disease, and congenital anomalies). In place of pulmonary functions, the parents should be given a diary card to record symptoms and precipitating events.

Treatment

The prevention of severe life-threatening attacks and the normalization of activity within the patient's life style are the primary goals in the therapy of asthma. A more ideal goal (though more difficult to realize) is the normalization of the patient's lung function. Toward these goals, every effort should be made to decrease the patient's baseline airways hyperreactivity and prevent it from increasing. The mainstay of the management of asthma is pharmacologic therapy. There are numerous books, symposia, and reviews detailing the basic pharmacology and clinical efficacy of the various pharmacologic agents used in asthma.[8,9,23–26] In this section both pharmacologic and nonpharmacologic therapy of asthma will be reviewed. The pharmacology relevant to the therapy of asthma for each drug class will be discussed and integrated into the treatment algorithms illustrated in the chapter (Appendixes 24.1–24.12). In addition, current treatment controversies and experimental therapies will be addressed. The treatment algorithms were developed by the National Asthma Education Program Expert Panel on the Management of Asthma from the National Heart, Lung, and Blood Institute.

In recent years, the increased awareness that inflammation plays a primary role in both the increased chronic bronchial hyperreactivity and the development of acute severe exacerbations in asthma has led to a change in the focus of therapy from the symptomatic relief of bronchospasm to preventing and suppressing the underlying inflammation.[4,26] Thus current therapeutic options in asthma consist of rescue, prophylaxis, and suppressive therapy.[4] Some medications are principally used to rescue patients from acute exacerbations while others are primarily used for prophylaxis to prevent exacerbations. The newest approach is to use drugs that suppress the inflammatory response thereby reducing the degree of bronchial hyperreactivity and improving long-term control and outcome in asthma.

Nonpharmacologic Management

Patient education and the teaching of patient self-management skills should be the cornerstone of any treatment program.[4] There are a number of published self-management programs for children and adults available through local American Lung Association chapters as well as asthma treatment centers and nationally through the National Heart, Lung, and Blood Institute and the Asthma and Allergy Foundation of America. Asthma self-management programs have been shown to improve patient adherence to medication regimens, improve self-management skills, and improve utilization of health care services.[27] A recent study demonstrated that teaching of self-management skills combined with improved access to health care providers in a special asthma clinic for severe adult asthmatics significantly reduced both the hospital admission and hospital day use rates.[28]

Self-management programs instruct patients in the pathogenesis of asthma and the appropriate use of their medications but principally focus on teaching patients to recognize triggers for their asthma and how to recognize early signs of deterioration. Use of objective measurement of airflow obstruction with a home peak flow meter is integral to the success of such programs.[28]

In patients with known allergenic triggers for their asthma, allergen avoidance has resulted in an improvement in symptoms, a reduction in medications, and a decrease in bronchial hyperreactivity.[29] Relatively simple environmental controls for patients allergic to house dust such as removing carpeting from bedrooms and using plastic pillow and mattress covers can reduce symptoms and need for medications.[24] Therefore, obvious environmental triggers (i.e., animals) should be avoided; however, there is very little evidence that extensive environmental controls (i.e., home air filtering systems) are of any value.

The role of immunotherapy (i.e., allergy shots) in asthma, although a proven and accepted therapy for allergic rhinitis, is still controversial.[24,26,30] Some studies have shown that immunotherapy of patients with very specific allergy reduces the number of late asthmatic responses and decreases bronchial sensitivity to the allergen while others have shown no effect.[30] There is increasing evidence suggesting that there may be a role for immunotherapy in asthma treatment.[30]

Oxygen therapy is indicated in patients requiring emergency room therapy for acute severe asthma.[9,23] Oxygen reverses bronchial hyperreactivity induced by hypoxemia, as well as the hemoglobin desaturation produced by V_a/Q mismatching. Patients hospitalized with acute severe asthma should be given adequate maintenance hydration in order to mobilize secretions; however, excessive hydration should be avoided to prevent excessive lung fluid at a time when patients have inflammation and bronchial edema.

Table 24.2 Pharmacologic Responses to Sympathomimetic Stimulation

Tissue	*Receptor type*	*Response*
Airways	β_2	Bronchodilatation, increased ciliary beat, increased mucus production, and inhibition of histamine release from mast cells
	α	Bronchoconstriction?
Heart	β_1	Chronotropic, inotropic
	β_2	Chronotropic
Vasculature	β_2	Vasodilatation
	α	Vasoconstriction
Skeletal	β_2	Increased neuromuscular transmission muscle (tremor, increased strength of contraction
Uterus	β_2	Relaxation (tocolysis)
Metabolic	α, β_1	Glycogenolysis, lipolysis
	β_2	Gluconeogenesis, lactic acidemia, hypokalemia
Mast cells	α	Augment mediator release
	β_2	Inhibit mediator release

Pharmacologic Management

β_2-Adrenergic Agonists

The β_2-adrenergic agonists are the most effective bronchodilators available today. β_2-Adrenergic receptor stimulation activates adenyl cyclase which produces an increase in intracellular cyclic AMP.[8,23] This increase results in smooth muscle relaxation, mast-cell membrane stabilization, and skeletal muscle stimulation. β_2-Adrenergic stimulation also activates Na^+, K^+ ATPase, produces gluconeogenesis, and enhances insulin secretion. These three effects combine to produce a mild to moderate decrease in serum potassium concentration by driving potassium intracellularly. The chronotropic response to β_2 agonists is mediated in part by baroreceptor reflex mechanisms as a result of the drop in blood pressure from vascular smooth muscle relaxation and direct stimulation of cardiac β_2 receptors as well as some β_1 stimulation at high concentrations.[31,32] Table 24.2 lists the pharmacologic effects of adrenergic receptor stimulation. Because their excessive cardiac stimulation produces cardiac arrhythmias and the inotropic effect enhancing myocardial oxygen consumption leads to myocardial necrosis, there is no rationale for using non–β_2-selective agonists in the treatment of asthma.[8,23,31]

Table 24.3 compares the various β-adrenergic agonists in terms of selectivity, potency, oral activity, and duration of action. The β_2 agonists are functional or physiologic antagonists in that they relax airway smooth muscle regardless of the mechanism for constriction.[8,23] When administered in equipotent β_2-agonist doses, all the drugs will produce the same intensity of response; the only differences will be in duration of action and cardiac toxicity. All of the β agonists are more bronchoselective when administered by the aerosol route.[32] Differences in myocardial effects are discernible between selective and nonselective agents even when administered as aerosols, particularly at the higher doses used for acute severe asthma.[31]

The aerosol administration of β_2 agonists not only enhances bronchoselectivity but provides a more rapid response and a greater degree of protection against provocations that induce bronchospasm such as exercise and allergen challenges than does systemic administration.[26,31] Currently, the only disadvantages to aerosol administration of β_2 agonists are the complexity of administration and the short duration of protection (Table 24.3). The latter problem may be solved by the introduction of the two newer β_2 agonists, formoterol and salmeterol, which have provided long-acting protection (8 to 12 hours) when administered as aerosols in preliminary clinical investigations. Because aerosol administration is so important to the use of β_2 agonists

Table 24.3 Relative Selectivity, Potency, and Duration of Action of the β-Adrenergic Agonists

	Selectivity			*Duration of action*		
Agent	β_1	β_2	β_2 *potency*[a]	**Bronchodilation (h)**	**Protection[b] (h)**	*Oral activity*
Isoproterenol	++++	++++	1	0.5–2	0.5–1.0	No
Metaproterenol	+++	+++	15	3–4	1–2	Yes
Isoetharine	++	+++	6	0.5–2	0.5–1.0	No
Albuterol	+	++++	2	4–8	2–4	Yes
Bitolterol	+	++++	5	4–8	2–4	No
Pirbuterol	+	++++	5	4–8	2–4	Yes
Terbutaline	+	++++	4	4–8	2–4	Yes
Formoterol	+	++++	0.24	8–12	8–12	Yes
Salmeterol	+	++++	0.50	8–12	ND[c]	UK[d]

[a] Relative molar potency: 1 = most potent.
[b] Protection refers to the duration of time that bronchoconstriction may be prevented.
[c] ND, not determined.
[d] UK, unknown.

Compiled from References 8, 9, 23, and 31.

and other antiasthmatics, there will be a discussion of the various aerosol delivery techniques at the end of this section.

The dose–response relationship for β_2-agonist-induced bronchodilation has been extensively studied and two aspects have significant clinical relevance. Both the intensity and duration of response are dose dependent, and more importantly the dose–response is a dynamic relationship. At increasing levels of baseline bronchoconstriction (irrespective of the stimulus), the dose–response curve is shifted to the right and the duration of bronchodilation is decreased.[23,31] This is reflected in the need for higher, more frequent doses in acute asthma exacerbations and why the duration of protection against significant provocation is much less than the duration of bronchodilation in chronic stable asthma (Table 24.3). The ability to increase the dose of aerosolized β_2 agonists by as much as fivefold to tenfold over those doses producing adequate bronchodilation in chronic stable asthmatics is what contributes to their efficacy in reversing the bronchospasm of acute severe asthma.

Besides the relatively short duration of protection against bronchoprovocation, β_2 agonists do not inhibit the late asthmatic response to allergen challenge or the subsequent bronchial hyperresponsiveness.[8] Long-term administration of β_2 agonists does not reduce bronchial hyperreactivity. Some studies have suggested that chronic β_2-agonist administration may actually increase bronchial hyperreactivity.[8] However, the mean change in bronchial hyperreactivity as measured by histamine or methacholine inhalation challenges did not exceed the 95% confidence interval for the reproducibility of these tests over time (i.e., within one to two doubling concentrations).[33] In addition, studies providing individual data indicate that the apparent increased reactivity does not occur in all patients, is not consistent over time with continuing treatment, nor is it a consistent finding in all studies.[8]

It has been suggested that the apparent increase in bronchial hyperreactivity may be a result of tachyphylaxis or tolerance produced by chronic β_2-agonist administration.[23,26] Chronic administration of β_2 agonists can lead to a down-regulation (decreased number of β_2 receptors) and a decreased binding affinity for these receptors.[31] Glucocorticoid therapy can both prevent and reverse this phenomenon.[26,31] Studies of β_2 receptors in vivo have primarily utilized surface β_2 receptors in lymphocytes, which may not reflect what occurs with respiratory smooth muscle β_2 receptors. A recent study has confirmed the development of tolerance to the extrapulmonary effects but not the bronchodilator response to chronic high-dose inhaled β_2 agonists.[34] The body of literature suggests that chronic β_2-agonist administration may produce a small degree of tolerance of minimal clinical significance that is easily overcome by increasing the dose or by administering glucocorticoids.

Clinical Use Inhaled selective β_2 agonists are indicated for the treatment of intermittent episodes of bronchospasm and are the bronchodilator as well as the first treatment of choice for acute severe asthma.[35,36] In acute severe asthma, β_2 agonists should be given in high doses by jet nebulization in frequent intervals (Table 24.4). Initially the patient should receive nebulizations every 20 minutes for the first 1 or 2 hours and then the dosage should be adjusted based on response (see treatment algorithms). During the recovery phase, the dose is generally lowered first and then the dosing interval is extended.

The inhaled selective β_2 agonists are the treatment of choice for EIA.[19] They inhibit EIA in a dose-dependent fashion and provide complete protection for a 2-hour period following inhalation with varying levels of patient-dependent protection over 4 hours.[9,19] The inhaled route is significantly more effective than the oral route of administration, which provides a moderate blocking effect similar to that seen for oral theophylline.[32]

The duration of action of the currently available inhaled β_2 agonists limits their usefulness in those patients who require chronic maintenance bronchodilators to prevent and control symptoms, particularly for those patients suffering from nocturnal asthma.[23] These patients can be treated with an oral sustained-release β_2 agonist. The need for chronic bronchodilator therapy may be an indicator of inadequate anti-inflammatory treatment and a dosage adjustment of these agents should be considered.

Methylxanthines

Methylxanthines have been used for asthma therapy for 50 years. Theophylline is the primary methylxanthine of interest, although others such as caffeine, dyphylline, and enprophylline also produce bronchodilation.[23] Caffeine and dyphylline are less potent than theophylline, while enprofylline, which is available in Europe, has greater bronchodilator potency.[24] Like the β_2 agonists, the methylxanthines are functional antagonists; however, their clinical potency is limited by their low therapeutic index.[23] Methylxanthines are ineffective by aerosol and therefore must be taken systemically. Theophylline as a sustained-release product is the preferred oral preparation, whereas its complex with ethylenediamine (aminophylline) is the preferred injectable product.

The mechanism by which theophylline produces bronchodilation is unknown but may involve inhibition of the release of intracellular calcium.[8,23] Theophylline is a competitive antagonist of bronchoconstrictor adenosine; however, this property is not shared by enprofylline, a more potent bronchodilator than theophylline.[23] Theophylline also stimulates endogenous catecholamine release.[8,23] These latter two effects are important determinants of toxic symptoms of excess theophylline. Both bronchodilation and protection against bronchoprovocation challenges are concentration dependent. Theophylline produces linear increases in bronchodilation with logarithmic increments in serum drug concentrations.[8,23]

The majority of chronic stable asthmatics will obtain significant bronchodilation when the serum theophylline concentration reaches 5 μg/mL and most patients will have no toxic symptoms with serum concentrations less than 15 μg/mL.[23] The percentage of patients experiencing adverse effects is approximately 18% at serum concentrations between 15 and 20 μg/mL.[23] This increases sharply to 60% at concentrations between 20 and 30 μg/mL, and 80% at concentrations greater than 30 μg/mL.

As with the β_2 agonists, the dose–response curves for smooth muscle relaxation by theophylline are dynamic and

Table 24.4 Dosages of Medications for Acute Severe Asthma

Medication	*Dosage* Pediatric	Adult	*Comment*
Sympathomimetics			For optimal delivery dilute aerosols to minimum of 4 mL maximum 6 mL; gas flow at 6–12 L/min
Isoetharine	0.1%–1.0% 0.1–0.2 mg/kg every 20 min for 3 doses, then every 1–2 h as needed	3–10 mg every 20 min for 3 doses then every 1–2 h as needed	Not recommended due to low potency and short duration
Metaproterenol 5% (50 mg/mL), 15 μg unit dose	0.25–0.5 mg/kg every 2–4 h as needed, maximum 15 mg	15 mg every 20 min for 3 doses, then 15–30 mg every 2–4 h as needed	Do not exceed maximum; not recommended in high dose due to lack of β_2 selectivity
Terbutaline Injection (1 mg/mL) Nebulizer solution (10 mg/mL)	0.1–0.3 mg/kg every 20 min for 3 doses, then every 2–4 h as needed	10 mg every 20 min then 10 mg every 2–4 h as needed	Currently not approved for this mode of administration; no advantage over albuterol so not recommended
Albuterol (5 mg/mL)	0.05–0.15 mg/kg every 20 min for 3 doses, then 0.15–0.3 mg/kg up to 10 mg every 2–4 h as needed, or 0.5 mg/kg/h by continuous nebulization	5–10 mg every 20 min or for 3 doses every 2–4 h as needed, or 10–15 mg/h by continuous nebulization	May continue every 20 min for 2–4 h in severe cases
Systemic			No proven advantage of systemic therapy over aerosol in patients capable of moving air
Epinephrine 1:1000 (1 mg/mL)	0.01 mg/kg up to 0.5 mg every 20 min for 3 doses SQ	0.3–0.5 mg every 20 min for 3 doses	
Sustained-action susphrine 1:200 (5 mg/mL)	0.005–0.01 mL/kg every 6–10 h as needed SQ	0.5–0.75 mg every 6–10 h as needed	
Terbutaline (1 mg/mL)	0.01 mg/kg every 20 min for 3 doses, then every 2–6 h as needed SQ	0.25–0.5 mg every 20 min for 3 doses, then every 2–6 h as needed	
	10 μg/kg over 10 min intravenously followed by 0.4 μg/kg/min. Increase as necessary by 0.2 μg/kg/min up to 3–6 μg/kg/min	Not recommended	Due to cardiac toxicity high dose inhaled agonists preferred
Anticholinergics Aerosol Atropine sulfate	0.05–0.075 mg/kg every 4–6 h as needed	0.025 mg/kg or 2.5–5 mg every 4–5 h as needed	Due to excellent absorption atropine sulfate not recommended
Ipratropium bromide 0.025%	250 μg every 4–6 h as needed	250–500 μg every 4–6 h as needed	
Glycopyrrolate (Robinul) 0.2 mg/mL injection	0.025–0.05 mg/kg nebulized every 4–6 h	2 mg nebulized every 2–6 h as needed	

Table 24.4 Dosages of Medications for Acute Severe Asthma (continued)

Medication	Dosage: Pediatric	Dosage: Adult	Comment
Glucocorticoids			
Methylprednisolone	1–2 mg/kg every 6 h for 24–48 h or severe symptoms abate, then reduce to 1–2 mg/kg/d every 12 h	80–200 mg/d in 2–4 divided doses	Duration of steroid therapy is dependent on response, continue full dose until patient at least 70%–75% of normal predicted FEV_1; hydrocortisone produces greater sodium retention; no advantage of parenteral therapy
Hydrocortisone	4 mg/kg every 4–6 h for 24–48 h, then reduce	200–400 mg/d 2–4 divided doses	
Prednisone	1–2 mg/kg/d in 2–3 doses for outpatient use for 3–5 days. Inpatient same as for methylprednisolone	40–160 mg/d in 2–4 divided doses	
Methylxanthines	See Fig. 24.6		Monitor theophylline serum concentration

shifted to the right in the face of increasing contractile stimuli.[23] This probably explains theophylline's relative lack of bronchodilatory effect in acute severe asthma.[25] The severity of theophylline's toxicity precludes even doubling the usual dosage.

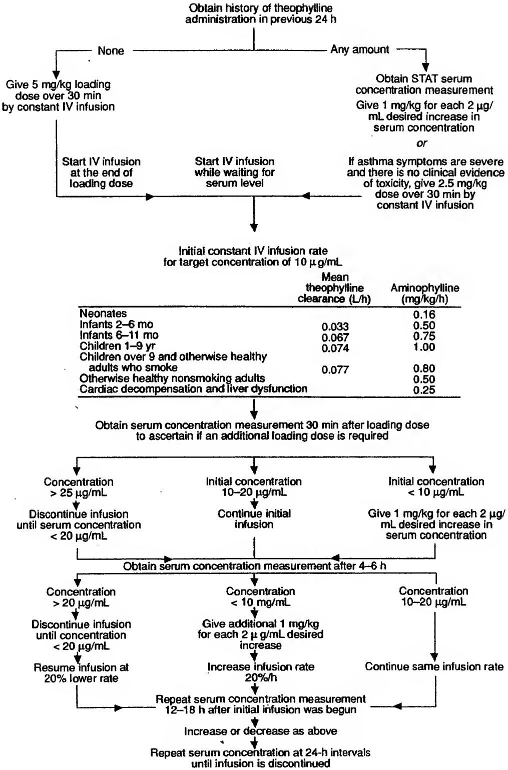
Theophylline has other effects that may be important to its antiasthmatic action. Theophylline inhibits pulmonary edema by decreasing vascular permeability, enhances mucocilary clearance, and strengthens contraction of a fatigued diaphragm.[23] In vitro theophylline inhibits the release of histamine in sensitized human lung fragments but has provided an inconsistent protection against the early asthmatic response to allergen.[23] When present in therapeutic concentrations, theophylline and enprophylline attenuate the bronchospasm of the late asthmatic response,[23] but has no apparent effect on the inflammation and subsequent increase in bronchial hyperresponsiveness.[26] Long-term administration does not reduce bronchial hyperreactivity.[26]

Other Effects Theophylline stimulates the central nervous system through its adenosine antagonism and produces cerebral vasoconstriction.[23] Both effects contribute to the neurotoxicities seen with theophylline. Theophylline acts as a respiratory stimulant by enhancing the hypoxic ventilatory drive, it decreases the lower esophageal sphincter pressure and increases gastric acid secretion, and has both inotropic and chronotropic cardiac effects.[26] Acutely theophylline acts as a diuretic but tolerance develops rapidly.

Pharmacokinetics An understanding of the pharmacokinetics combined with routine monitoring of serum concentrations is essential for the safe and effective use of theophylline. Theophylline is primarily eliminated by metabolism via the hepatic cytochrome P-450 mixed-function oxidase microsomal enzymes with 10% or less excreted unchanged in the kidney.[23] Each of the major metabolic pathways for theophylline is saturable within the usual therapeutic concentration so that theophylline frequently, though not always, exhibits nonlinear pharmacokinetics.[23] This may partially explain the relatively large intrapatient variability in theophylline clearance (often as great as 30%) over time.[32] Part of the intrapatient variability in clearance is age dependent with 1 to 9 years olds having the greatest clearance rates, and therefore requiring the largest dosages for theophylline (Fig. 24.8). However, even within the same age groups theophylline clearance rates can vary twofold to threefold so that no patient should be treated with theophylline without routine monitoring of serum theophylline concentrations. Figures 24.8 and 24.9 and Table 24.5 give recommended dosages, monitoring schedules, and dosage adjustments for intravenous aminophylline and oral theophylline.

The hepatic P-450 enzymes are susceptible to induction and inhibition by various environmental factors and drugs. These are listed in Table 24.6. Only those drugs that produce a 20% or greater inhibition or 50% or greater induction of theophylline metabolism are likely to result in clinically significant interactions. Also, there is a significant interpatient susceptibility to developing an interaction even with potent P-450 inhibitors such as cimetidine. However, the clinician needs to be aware of drugs that have been proven to alter theophylline metabolism, or could potentially do so, in order to provide appropriate alternatives and monitor the patient appropriately.

Due to the relatively short elimination half-life of theophylline (3 to 5 hours in children and 6 to 12 hours in adults), sustained-release oral preparations are favored for outpatient therapy. These preparations can be administered every 8 to 24 hours in patients and maintain relatively constant therapeutic serum concentrations, and the decreased dosing frequency improves compliance.[24] The degree of serum theophylline concentration fluctuation over the dosing inter-



	Mean theophylline clearance (L/h)	Aminophylline (mg/kg/h)
Neonates		0.16
Infants 2–6 mo	0.033	0.50
Infants 6–11 mo	0.067	0.75
Children 1–9 yr	0.074	1.00
Children over 9 and otherwise healthy adults who smoke	0.077	0.80
Otherwise healthy nonsmoking adults		0.50
Cardiac decompensation and liver dysfunction		0.25

Figure 24.8 Algorithm for the use of theophylline to relieve acute symptoms of asthma. Aminophylline = 80% theophylline. *(From Jenne JW, Murphy S [eds]. Drug Therapy for Asthma: Research and Clinical Practice. New York, Marcel Dekker, 1987, with permission.)*

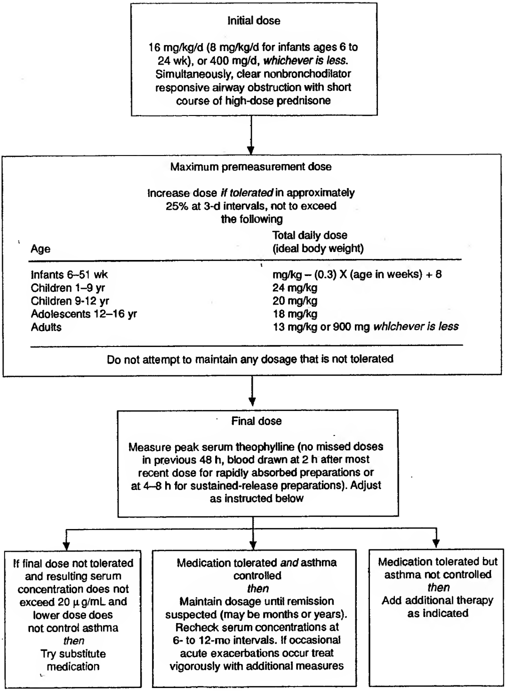


Figure 24.9 Algorithm for the use of theophylline to prevent symptoms of chronic asthma. Doses are expressed and 24-hour total amounts should be divided into two or three equal intervals depending on the product and patient. *(From Jenne JW, Murphy S [eds]. Drug Therapy for Asthma: Research and Clinical Practice. New York, Marcel Dekker, 1987, with permission.)*

val is dependent on the release characteristics of the products as well as the elimination rate characteristics of the patients.[23] Thus patients with rapid clearance rates for theophylline will experience greater fluctuations than patients with slow clearance rates, given the same product over the same dosing interval.[23] Neither an optimal nor an acceptable maximum fluctuation has been absolutely established for theophylline serum concentrations but it seems reasonable that it should not exceed the usual therapeutic range. That is, it should not exceed 100% where

$$\%\ \text{fluctuation} = \frac{C_{\max} - C_{\text{pmin}}}{C_{\text{pmin}}}$$

Table 24.5 Dosage Adjustment After Serum Theophylline Measurement

If serum theophylline is		*Directions*
Within normal limits	10 to 20 μg/mL	Maintain dosage if tolerated. Recheck serum theophylline concentration at 6- to 12-month intervals[a]
Too high	20 to 25 μg/mL	Decrease doses by about 10%. Recheck serum theophylline concentration at 6- to 23-month intervals[a]
	25 to 30 μg/mL	Skip next dose and decrease subsequent doses by about 25%. Recheck serum theophylline
	Over 30 μg/mL	Skip next 2 doses and decrease subsequent doses by 50%. Recheck serum theophylline
Too low	7.5 to 10 μg/mL	Increase dose by about 25%.[b] Recheck serum theophylline concentration at 6- to 12-month intervals[a]
	5 to 7.5 μg/mL	Increase dose by about 25% to the nearest dose increment[b] and recheck serum theophylline for guidance in further dosage adjustment (another increase will probably be needed, but this provides a safety check)

[a] Finer adjustments in dosage may be needed for some patients.
[b] Dividing the daily dose into 3 doses administered at 8-hour intervals may be indicated if symptoms occur repeatedly at the end of a dosing interval.

From Jenne JW, Murphy S (eds). Drug Therapy for Asthma: Research and Clinical Practice. New York, Marcel Dekker, 1987, with permission.

Each of the sustained-release theophylline products has different release characteristics and the products are variably susceptible to altered absorption from food or gastric pH changes.[24] Preparations with slower release characteristically exhibit a significant diurnal variation in absorption with the rate significantly slower at night in the recumbent patient.[23] As a result of these differences, it is best not to consider the sustained-release preparations interchangeable. In general, preparations unaffected by food that can be administered a minimum of every 12 hours in most patients are preferable.

Clinical Use In the 1970s and 1980s theophylline was a primary drug for the treatment of both acute and chronic asthma in the United States. However, the availability of safer more effective inhaled β_2 agonists and anti-inflammatory agents (cromolyn and topically active inhaled glucocorticoids) coupled with a better understanding of the pathogenesis of asthma and bronchial hyperreactivity, has curtailed the application of theophylline.

A series of investigations in the 1980s evaluating the therapy of acute asthma in the emergency room demonstrated that theophylline did not add to the efficacy of aerosolized β_2 agonists but frequently increased toxicity.[35,36] The value of theophylline in the hospitalized patient was less clearly delineated because those studies that evaluated patients for at least 24 hours tended to show a positive benefit for theophylline.[37] However, a recent double-blind placebo-controlled trial of aminophylline for the hospitalized asthmatic failed to detect any benefit of adding aminophylline to inhaled β_2 agonists and oral glucocorticoids.[38]

In the outpatient setting chronic theophylline administration can reduce asthma symptoms, reduce the amount of as-needed inhaled β_2 agonists used, and reduce the oral

Table 24.6 Factors Affecting Theophylline Clearance

Decreased clearance	*% decrease in clearance*[a]	*Increased clearance*	*% increase in clearance*[a]
Cimetidine	−35 to −60	Rifampin	+53
Troleandomycin	−50	Carbamazepine	+50
Erythromycin	−25	Phenobarbital	+34
Allopurinol	−20	Phenobarbital	+95
Propranolol	−30	Phenytoin	+70
Oral contraceptives	−10 to −30	Smoking	+40
Enoxacin	−65	High-protein diet	+25
Ciprofloxacin	−25 to −30	Charcoal broiled meat	+30
Norfloxacin	−10	Intravenous isoproterenol	
Ofloxacin	−26	Sulphinpyrazone	+22
Febrile viral illness	−50		

[a] Approximate means reported across studies.

From References 23 and 25.

steroid requirement in steroid-dependent asthmatics.[24] Sustained-release theophylline provides significantly less protection but longer duration against bronchoconstrictive stimuli than currently available inhaled β_2 agonists.[24] This is particularly important for patients with nocturnal asthma.[26] On the other hand, comparative studies between sustained-release theophylline and oral sustained-release β_2 agonist have not shown any advantage for theophylline.[32,39] Significant disadvantages to chronic theophylline therapy include theophylline's lack of effect on underlying bronchial hyperreactivity and the dangers inherent in giving a drug that can produce severe neurologic toxicity, including seizures, permanent neurologic deficit, and death at serum concentrations only twofold greater than optimal therapeutic concentrations. Death has occurred in children receiving their usual doses of theophylline during acute febrile viral illnesses.[23] A review of studies comparing cromolyn and theophylline as first-line therapy for chronic asthma failed to find an advantage for theophylline over cromolyn.[40]

Serum theophylline concentrations must be routinely monitored for the safe and effective use of theophylline. The usually accepted therapeutic range of 10 to 20 μg/mL is not an absolute but a statistical concept. Many patients will respond to lower concentrations. A range of 5 to 15 μg/mL may well be as effective and a safer range of steady-state concentrations for most patients. Due to the log-linear nature of the concentration–response curve, there is little to gain in terms of bronchodilation by going from 15 μg/mL to 20 μg/mL.[23] Patients whose theophylline concentrations are maintained near 20 μg/mL are more susceptible to developing serious adverse effects when confronted with an environmental exposure that inhibits theophylline metabolism. Due to its high risk:benefit ratio theophylline should be considered as a second- or third-line drug in the therapy of asthma.

Anticholinergics

The anticholinergic agents have been used for centuries in the form of stramonium herbal treatments for asthma.[23] However, their systemic effects, particularly involving the central nervous system, limited their usefulness. The recent introduction of quaternary ammonium derivatives such as ipratropium bromide (Atrovent) has renewed interest in these compounds.[26] Anticholinergic bronchodilators are competitive inhibitors of muscarinic receptors.[23] Unlike β_2 agonists and theophylline, they are not functional antagonists; they only produce bronchodilation in cholinergic-mediated bronchoconstriction. Normal bronchial tone is maintained through parasympathetic innervation of the airways via the vagus nerve. A number of the triggers and mediators of asthma (i.e., histamine, prostaglandins, sulfur dioxide, exercise, allergens) produce bronchoconstriction in part through vagal reflex mechanisms.[8,23] Studies of asthmatics consistently demonstrate that anticholinergics are effective bronchodilators though not as potent as β_2 agonists. Anticholinergics attenuate but do not block allergen- or exercise-induced asthma in a dose-dependent fashion and have no effect on the late asthmatic response.[23] A recent review found that anticholinergics consistently produce bronchodilation in acute severe asthma.[41] Most studies suggest that anticholinergics can be expected to produce a further 20% to 25% improvement in FEV_1 over β_2 agonists alone.[41] However, a significant interpatient variability exists with some patients obtaining significantly greater (40% to 80%) improvements, and others minimal improvement.

Currently available anticholinergics are nonselective muscarinic receptor blockers, and blockade of inhibitory muscarinic receptors could theoretically result in an increased release of acetylcholine and overcome the block on the smooth muscle receptors (M_3).[26] This mechanism may explain why some patients have experienced paradoxical bronchoconstriction from nebulized anticholinergics. All anticholinergics are somewhat selective in that bronchodilation occurs at a lower dose than is required to dry secretions or produce tachycardia.[23] As with the β_2 agonists, anticholinergic bronchoselectivity is increased by aerosol administration.

Atropine sulfate is a tertiary ammonium compound that is completely absorbed from the lungs and gastrointestinal tract.[23,41] It has an elimination half-life of 3 to 4 hours in young adults which is prolonged in young children and the elderly who may accumulate toxic concentrations with continued dosing.[23] The quaternary ammonium derivatives (ipratropium bromide, atropine methonitrate, oxitropium, and glycopyrrolate) have the advantage of poor absorption across mucosae and the blood–brain barrier. This results in negligible systemic effects with a prolonged local effect (i.e., bronchodilation). In addition, the quaternary compounds appear not to produce the decrease in mucociliary clearance seen with the tertiary compounds (atropine and hyoscine).[23] Table 24.7 compares the anticholinergic agents.

The anticholinergics have a longer duration of action than the currently available β_2 agonists. Both intensity and duration of action are dose dependent. Time to reach maximum bronchodilation from aerosolized anticholinergics is considerably slower than from aerosolized β_2 agonists (2 hours versus 30 minutes). However, this is of little clinical consequence as some bronchodilation is seen within 30 seconds, 50% of maximum response occurs within 3 minutes, and 80% of maximum is reached within 30 minutes.[41]

Clinical Use The role of anticholinergics in the treatment of asthma is still undefined. Studies in chronic bronchitis, a disease in which increased parasympathetic action is the predominant reversible component, show that anticholinergics produce similar bronchodilation to β_2 agonists with fewer side effects. However, anticholinergics are unable to produce maximum bronchodilation in asthma and in the usual recommended doses are less effective than the β_2 agonists.[23] Although they produce additive bronchodilation to β_2 agonists and/or theophylline in chronic asthma, the significance of this is unknown. There is not a significant tolerance produced with prolonged administration nor is there any effect on bronchial hyperreactivity.[8] Both the β_2 agonists and cromolyn provide superior protection against EIA.[23]

Most studies indicate that anticholinergics produce additive bronchodilation with β_2 agonists in acute severe asthma.[41] However, the effect is inconsistent and even in those studies demonstrating significant further bronchodilation, the overall outcome of the patients (i.e., number admitted to hospital or length of hospital stay) was not altered. Despite these results, anticholinergics may represent a safer and

Table 24.7 Comparison of Anticholinergics[a]

Agent	Availability	Relative potency[b]	Duration (h)	Dosages: Pediatrics	Dosages: Adult
Atropine sulfate	Ophthalmic solution 0.5% (5 mg/mL) 1.0% (10 mg/mL)	1	3–5	0.05 mg/kg nebulized q 4–6 h	2.5–5.0 mg nebulized q 6 h
Atropine methonitrate	Investigational only	0.5	5–6	ND[c]	1.5 mg nebulized q 6 h
Ipratropium bromide (Atrovent)	MDI[d] 18 μg/actuation 300 actuations .025% (0.25 mg/mL) nebulizer solution	Unknown	5–6	2 inhalations q 6 h nebulized 250 μg nebulized q 4–6 h for acute asthma	2–4 inhalations q 6 h or 500 μg nebulized q 6 h for acute asthma
Glycopyrrolate (Robinul)	0.2 mg/mL injectable solution	0.5	6–12	0.025–0.05 mg/kg nebulized q 6 h	2 mg nebulized q 6 h
Oxitropium bromide	Investigational	Unknown	8	ND[c]	500 μg

[a] At the current time there are no anticholinergics approved by the FDA for use in asthma.
[b] Dose required to produce equivalent bronchodilation.
[c] ND, not determined.
[d] MDI, metered-dose inhaler.

Compiled from References 8, 10, 23, and 41.

more effective additional bronchodilator than theophylline in this setting. Anticholinergics appear to be as effective as β_2 agonists for reversing the bronchospastic component of acute exacerbations of chronic bronchitis.[23,41]

Cromolyn Sodium

Cromolyn sodium or disodium cromoglycate has been available for the prophylactic treatment of asthma for almost 20 years and its exact mechanism of action is still unknown. Initially, it was thought that all of its activity was a result of mast cell membrane stabilization. As such, it inhibits the early asthmatic response to allergen challenge as well as EIA.[40] Unlike the β_2 agonists and other experimental mast cell membrane stabilizers, cromolyn also inhibits the late asthmatic response and prevents the subsequent increased bronchial hyperreactivity.[8,26] Long-term prophylaxis with cromolyn prevents the usual rise in bronchial hyperreactivity associated with specific pollen seasons and may produce a modest decrease in baseline bronchial hyperreactivity.[8,40] These findings suggest that cromolyn also has an inhibitory effect on other inflammatory cells such as macrophages and eosinophils.[26] Cromolyn also inhibits neurally mediated bronchoconstriction mediated through C-fiber sensory nerves in the airways.[26] Similar to chronic therapy with glucocorticoids, cromolyn is capable of preventing both the early and late asthmatic response. Cromolyn has no bronchodilatory effect.

Cromolyn is only effective by inhalation and is available as a metered-dose inhaler (MDI), dry powder inhaler, and a nebulized solution. Cromolyn is not bioavailable orally but the portion of the dose that reaches the lung is completely absorbed.[40] Absorption from the airway is significantly slower than elimination (1–2 hours versus 0.32 hours). Both the intensity and duration of protection against various challenges is dose dependent.[40] Higher doses produce greater and more prolonged protection.

Cromolyn is remarkably nontoxic. No evidence of mutagenesis or teratogenesis has been found. Less than 0.1% of an intravenous dose crosses the placenta or enters breast milk. Rarely have allergic reactions been reported in patients taking the dry powder inhaler.[40] Many of these were a result of the lactose in the formulation that is not found in the MDI or nebulizer solution. Cough and wheeze has also been reported following inhalation of the dry powder inhaler but not the other formulations. Cromolyn is undoubtedly the least toxic drug used to treat asthma with significant adverse effects occurring in less than 1 in 10,000 patients.[8,23,40] Tolerance to cromolyn has not been demonstrated.

Approximately 60% to 75% of patients (adults and children) with mild to moderate chronic asthma will be adequately controlled with cromolyn.[40] Comparative studies with theophylline do not demonstrate a significant advantage for either agent in controlling symptoms of asthma or improving baseline pulmonary functions.[40] However, cromolyn produced a significant decrease in bronchial hyperreactivity and theophylline did not,[40] and theophylline therapy may produce more side effects and require more patient visits for monitoring.[40]

Clinical Use Cromolyn is indicated for the prophylaxis of chronic mild to moderate asthma in both children and adults regardless of etiology. Cromolyn is particularly effective for the allergic asthmatics on a seasonal basis or just prior to an acute exposure (i.e., animals or mowing the lawn). Cromolyn is the second drug of choice for the prevention of EIA and may be used in conjunction with a β_2 agonist in more severe cases not completely responding to either agent alone. In those patients with a history of a late asthmatic response following exercise, cromolyn would be the first choice.[40] Many authors consider cromolyn to be the anti-inflammatory of first choice for childhood asthma due to its efficacy and safety.[8,26,40]

The efficacy of cromolyn is directly related to its degree of deposition in the lung so when beginning cromolyn therapy,

it is important that the airways are patent. A short course of systemic glucocorticoids and around-the-clock inhaled β_2 agonists may initially be required in patients with significant obstruction. Most patients will experience an improvement in 1 to 2 weeks but it may take longer to achieve maximum benefit. Patients should initially receive cromolyn four times daily and then only after stabilization of symptoms may the frequency be reduced to two to three times daily. It is not necessary to maintain the regular use of concomitant β_2 agonists after the patient becomes stable; they can be reduced as needed.

Nedocromil sodium, a pyranoquinoline dicarboxylic acid that is pharmacologically similar to cromolyn, is available in Europe and will soon be available in the United States for the prophylaxis of asthma. Nedocromil appears to have greater molar potency than cromolyn, but whether or not this will translate into greater efficacy awaits comparative trials.[8,23] Like cromolyn, nedocromil is administered by inhalation 4 mg two to four times daily. It is not as effective as glucocorticoids at reducing bronchial hyperreactivity and like cromolyn has only marginal benefits in steroid-dependent asthmatics.[8]

Glucocorticoid Therapy

General Characteristics

Recent evidence that intensive pharmacotherapy can alter the level of hyperreactivity has reestablished the utility of glucocorticoid therapy for asthma.[26] The mechanism of action and use of glucocorticoids in asthma have been recently reviewed.[42] Actions useful in treating asthma include (1) increasing the number of β-adrenergic receptors and improving the receptor responsiveness to β-adrenergic stimulation (glucocorticoids also restore and prevent tolerance induced with chronic administration of β-adrenergics); (2) reducing mucus production and hypersecretion; and (3) inhibiting the inflammatory response at all levels. Glucocorticoids constrict the microvasculature inhibiting fluid and protein influx, and inhibit migration of neutrophils and eosinophils into tissues as well as inhibiting their function.[42] Glucocorticoids inhibit the synthesis but not release of histamine from mast cells. Glucocorticoids inhibit the production of prostaglandins and leukotrienes by inhibiting phospholipase release of arachidonic acid from membrane phospholipids.[42]

Cortisol and its synthetic derivatives such as prednisolone, methylprednisolone, triamcinolone, dexamethasone, and betamethasone all have beneficial effects in the treatment of asthma related to the prevention or suppression of airway inflammation.[8,23,42] The major cellular and biochemical activity of the glucocorticoids include decreasing synthesis and release of proinflammatory mediators; reducing inflammatory cell activation, recruitment, and infiltration; and decreasing vascular permeability.[42] Suppressing the ongoing airways inflammation results in prevention or inhibition of mucus secretion, decreased edema of airway mucosa, and perhaps decreased airway epithelial denudation, leading to a reduction in airways reactivity.[42] What may be more clear is that airway epithelium appears to regenerate toward normal with ongoing therapy with inhaled glucocorticoids. Additional useful antiasthma effects of glucocorticoids include increasing the bronchial smooth muscle relaxing effect of β-receptor stimulation,[42] and thereby preventing or decreasing β-adrenergic tachyphylaxis that occurs with chronic usage.[42]

Time Course of Response

Glucocorticoids act through the production of lipocortin; therefore, the time required to see the particular effect is dependent on the time required for lipocortin synthesis, decreased formation of the particular mediator, and resolution of the response. Generally, the cellular and biochemical effects are immediate, but varying amounts of time are required to produce a clinical response. β-Receptor density increases within 4 hours of glucocorticoid administration.[42] Improved responsiveness to β agonists occurs within 2 hours.[43] In acute severe asthma (status asthmaticus), 4 to 12 hours may be required before any clinical response is noted, most likely due to the time required to produce sufficient anti-inflammatory response. Reversal of seasonal increased bronchial hyperreactivity requires at least 1 week of therapy.[42] Reactivity to EIA decreases after 4 weeks of therapy.[19] Although single doses do not inhibit the immediate asthmatic response to antigen challenge, continued therapy for 1 week partially suppresses the response.

Regardless of the type of glucocorticoid therapy, bronchodilator and/or cromolyn therapy should be used in conjunction to allow a decrease in the glucocorticoid dose required to control symptoms. High-dose systemic steroids should be administered to all steroid-dependent asthmatics during acute attacks. Aerosol glucocorticoids are not effective in acute asthma attacks.[8,43] There is no evidence that the use of glucocorticoids in the moderate asthmatic will induce a state of steroid dependence. In fact, most of the evidence demonstrating a decrease in bronchial hyperreactivity with steroid therapy implies just the opposite.

Systemic Glucocorticoid Therapy

Acute severe asthma, status asthmaticus, is treated with high-dose systemic glucocorticoids combined with frequent administration of inhaled β-adrenergic bronchodilator agents. Glucocorticoids can be administered by the parenteral route (methylprednisolone sodium succinate, hydrocortisone sodium succinate) or alternatively by the oral route (prednisone, methylprednisolone), which provide a rapid onset of action and a systemic effect.[43] The glucocorticoids used in asthma are compared in Table 24.8. Systemic steroids should be administered in a dose approximately equivalent to methylprednisolone 1 mg/kg intravenously or orally every 6 hours.[43] There is no difference in response to intravenous and oral administration. Following resolution of severe obstruction, the steroid dose is reduced and administered by the oral route. The duration of treatment is dependent on the patient's response and past history.

Glucocorticoids are also recommended for the treatment of impending episodes of severe asthma unresponsive to bronchodilator therapy.[43] Prednisone, approximately 1 to 2 mg/kg/d (up to 30 to 40 mg/dose), is administered orally in two divided doses for 3 to 7 days.[43] If an adequate response is not achieved, administration of prednisone three times daily may be worthwhile. Once again, the dose and duration of treatment is based on the patient's response and past

Table 24.8 Glucocorticoid Comparison Chart

Systemic	*Relative anti-inflammatory potency*	*Relative sodium-retaining potency*	*Duration biologic activity (h)*	*Plasma elimination half-life (h)*	*Equivalent dose (mg)*
Hydrocortisone	1	1	8–12	1.5–2	20
Prednisone	4	0.8	12–36	2.5–3.5	5
Prednisolone	4	0.8	12–36	2.5–3.6	5
Methylprednisolone	5	0.5	12–36	3.3	4
Triamcinolone	5	0	12–36	2.5–3.3	4
Betamethasone	25	0	36–54	5–7	0.75
Dexamethasone	25	0	36–54	3.4–4	0.75

Aerosol	*Relative topical potency*	*Systemic bioavailability (%)*	*Dosage per inhalation (μg)*	*Plasma elimination half-life (h)*
Beclomethasone-16,17-dipropionate (Forte)	0.3–0.5	<5	42 (250)	15
Budesonide[a]	1.0	10	50	2–2.8
Flunisolide	0.05	20	250	1.6
Triamcinolone-16,17-acetonide	0.2	Unknown	100	Unknown

[a] Investigational.

history. The effects of glucocorticoids in asthma are dose and duration dependent. This is true as well for the adverse effects of systemic steroids (Table 24.9). The clinician is continually forced to balance the toxicity of chronic systemic glucocorticoid therapy versus control of asthma symptoms. Because short-term (1 to 2 weeks) high-dose steroids (1 to 2 mg/kg/d methylprednisolone) do not produce serious toxicities, the ideal use is to administer the glucocorticoids in a short "burst" and then maintain the patient on bronchodilators and/or cromolyn with long periods between systemic glucocorticoid treatment. In general, glucocorticoid therapy for more than 5 days at doses that exceed the usual physiologic endogenous cortisol production will cause temporary aberration in adrenal cortisol release. However, in studies this hypothalamic–pituitary–adrenal (HPA) axis suppression is short lived (1 to 3 days) and readily reversible following short bursts (10 days or less) of pharmacologic doses of glucocorticoids.[43] There is probably a maximum number of short bursts a patient can receive after which chronic steroid side effects occur. Patients receiving at least eight bursts were shown to have a similar decrease in trabecular bone density as those patients on daily or alternate day steroids over 1 year.[43] Children who received four or more bursts of prednisone exhibited a subnormal response to hypoglycemic stress or adrenocorticotropic hormone (ACTH) administration.[43] Very short courses of glucocorticoids (3 to 5 days) have been effective in reducing hospitalization from acute exacerbations. Short-burst steroid therapy is often effective in reducing hospitalizations in moderate asthmatics.[43] In patients who require chronic systemic glucocorticoids for control of asthma, the lowest possible dose required to control symptoms is the goal of therapy. Physicians will often sacrifice complete control of the patient's symptoms to avoid toxicity. Two methods of decreasing the toxicities of systemic glucocorticoid therapy is to use alternate-day therapy or the topical inhaled glucocorticoids. In patients with poorly controlled chronic asthma, oral glucocorticoids may be administered in a dosing schedule similar to that previously described, to maximize pulmonary function. Once this goal is achieved, the prednisone dose is tapered and may be supplemented and eventually replaced by inhaled glucocorticoids.[8,9,23,42]

Table 24.9 Adverse Effects of Chronic Systemic Glucocorticoid Administration

Hypothalamic–pituitary–adrenal suppression
Growth retardation
Skeletal muscle myopathy
Osteoporosis fractures
Aseptic necrosis of bone
Pancreatitis
Pseudotumor cerebri
Psychiatric disturbances
Sodium and water retention
Hypokalemia alkalosis
Hypertension
Skin striae
Impaired wound healing
Inhibition of leukocyte and monocyte function
Subcutaneous tissue atrophy
Glaucoma
Posterior subcapsular cataracts
Moon facies
Central redistribution of fat

Inhaled Glucocorticoids

The inhaled glucocorticoids are becoming more popular in the United States as first-line therapy for chronic asthma. This is because the contribution of inflammation to the pathogenesis of asthma is becoming better understood and

the inhaled glucocorticoids allow the application of potent topical anti-inflammatory agents to the relevant site of action within the airways.[26] The glucocorticoids currently available for inhaled use are beclomethasone diproprionate, triamcinolone acetonide, and flunisolide (Table 24.8). Budesonide, widely used internationally, is not yet available in the United States.

Doses of inhaled glucocorticoids required to control moderate to severe asthma have been associated with the same degree of systemic effect as 15 mg/d of prednisone, yet bioequivalent with prednisone 60 mg/d in terms of anti-asthma potency.[42] Therefore, the inhaled glucocorticoids demonstrate a favorable topical:systemic potency ratio, but should not be considered benign. The "ideal" glucocorticoid for inhaled use should have a higher degree of topical potency, minimal systemic absorption of active drug, and minimal local or systemic side effects. None of the available inhaled glucocorticoids are considered ideal and ongoing investigation into topical:systemic potency ratios will reveal important and much needed information. The available inhaled glucocorticoids, beclomethasone dipropionate, triamcinolone acetonide, and flunisolide, appear to have relatively similar topical:systemic potency ratios; however, more clinical studies are needed to clarify issues of comparative efficacy and toxicity.[24,42] All three are administered by a metered-dose inhaler. Unfortunately, there is no glucocorticoid approved for nebulized administration in the United States.

The inhaled glucocorticoids have high topical anti-inflammatory effects and are metabolized to less active substances when absorbed. As with systemic glucocorticoid therapy, the lowest dose required to control symptoms is the appropriate dose. The inhaled glucocorticoids produce dose-dependent suppression of the adrenal cortex, but much less than systemic glucocorticoids.[42] Patients derive increased benefits from increasing the dose of beclomethasone dipropionate from 400 to 1600 μg daily. Measurable adrenal suppression occurs at dosages greater than 800 μg daily.[9] Daily aerosol glucocorticoid administration often produces greater control than alternate-day systemic glucocorticoids.[10] The combination can be used for further improvement; in addition, aerosol glucocorticoids may allow the systemic dose to be lowered in the severe steroid-dependent asthmatic.

Local adverse effects of aerosol glucocorticoids include oropharyngeal candidiasis and dysphonia that are dose dependent. The dysphonia appears to be due to a local steroid-induced myopathy of the vocal chords. The use of a spacer device can decrease oropharyngeal deposition and decrease the incidence and severity of local side effects.[9] Optimal dosing of aerosol glucocorticoids has not been thoroughly investigated. A number of patients may be controlled with bid dosing; however, a recent investigation demonstrated an improved asthma response with decreased systemic effects by giving the same total daily dose four times daily as opposed to twice daily.[23] So although twice-daily dosing may improve adherence, it may be at the cost of decreased efficacy and increased toxicity.

Regardless of the type of glucocorticoid therapy, bronchodilator therapy should be used in conjunction to allow a decrease in the glucocorticoid dose required to control symptoms. There is no evidence that the use of glucocorticoids in the moderate asthmatic will induce a state of steroid dependence. In fact, most of the evidence demonstrating a decrease in bronchial hyperreactivity with steroid therapy implies just the opposite.

At the present time, product information is clear regarding maximum dosing guidelines for adults and children with the available inhaled glucocorticoids. For beclomethasone, triamcinolone, and flunisolide, the maximum recommended dose for adults is equivalent to 1600 and 2000 μg/d, respectively. Therefore, patients requiring doses of the inhaled glucocorticoids approaching or exceeding 2000 μg/d should be monitored for adverse effects. These maximum dosing guidelines are even lower for children. Needless to say, more studies are needed in both adults and children to establish safety for doses that exceed the product recommendations. There is also a need for long-term studies to assess the safety and efficacy of inhaled glucocorticoids in children, particularly in regard to growth and development, bone metabolism, and other adverse effects.

Spacer Devices and Inhaled Glucocorticoids

The bioavailability of the inhaled glucocorticoids is influenced by the method of delivery, systemic absorption from the gastrointestinal tract and lung, and general factors affecting elimination of the drug. Deposition to the site of drug action within the airways can be substantially increased by application of spacer devices available from various manufacturers. This enhanced deposition will decrease asthma symptoms and improve spirometry in patients with moderate to severe asthma.[42] Coincident with the improvement in asthma is the decrease in frequency of colonization of the oropharynx with candida.[42]

Miscellaneous Therapies

Antihistamines

Antihistamines have had a controversial role in asthma therapy. Early studies demonstrating the role of histamine release in bronchoconstriction suggested a potential benefit of antihistamine therapy; however, studies in chronic and acute asthma did not support the initial enthusiasm.[44] The pendulum then swung in the opposite direction on the theoretical (but unproven) grounds that antihistamines through their anticholinergic and mucus-drying effect could be harmful in asthma. The use of anticholinergics without worsening asthma should finally put this concern to rest. Indeed, most studies of chronic and acute administration of antihistamines in asthma have demonstrated small improvement of symptoms and pulmonary functions or no effect.[44] While antihistamines are generally not helpful in asthma, they are not contraindicated. They are useful adjunctive therapy for the patient with allergic rhinitis and asthma.

Ketotifen Ketotifen (a benzocyclohepathiophene derivative) is a potent investigational antihistamine with in vitro antianaphylactic, SRS-A inhibition, and mast cell stabilizing properties undergoing extensive clinical testing.[25,44] Although initial trials suggested efficacy equivalent to cromolyn for EIA and antigen-induced asthma, recent controlled trials have shown that ketotifen's activity is primarily

due to its powerful antihistaminic effect.[44] As such, ketotifen has a modest effect in asthma and does not appear to be a significant addition to asthma therapy. Thus the initial enthusiasm for ketotifen as an orally active cromolyn-like prophylactic drug has waned somewhat. Further studies will be required before the efficacy of ketotifen in asthma is established.

Calcium Channel Blockers

The calcium channel blockers have engendered a great deal of interest because of the central role of calcium ion flux in smooth muscle contraction and membrane stabilization.[23] The calcium channel blockers do not produce significant bronchodilation although they do produce significant vasodilation.[23] It appears that the cardiovascular selectivity of the current calcium channel blockers may be due to binding or receptor differences. In vitro, similar effects in airway smooth muscle can be produced with doses 1000 times those required in vascular preparations.[23] Verapamil and nifedipine have only modest effects on EIA and histamine-induced bronchospasm, and have not been useful in the treatment of chronic asthma. They certainly are not contraindicated and should be considered over β-blockers in asthmatics with hypertension or arrhythmias responsive to either.

Methotrexate

Similar to the success of low-dose methotrexate in the treatment of psoriatic and rheumatic disorders, the use of this agent in the treatment of severe asthma has met with some success in controlled studies.[45] In these trials, methotrexate was not used alone, rather as a "glucocorticoid-sparing" agent. Whether methotrexate would have efficacy when used without systemic glucocorticoids in patients with milder asthma is not known. Although its primary mechanism of action is not understood, methotrexate may be acting as an anti-inflammatory agent.[8] Methotrexate may also have immunomodulatory effects at very low and relevant concentrations in vitro.[8] The potential beneficial effects of methotrexate has raised questions whether other chemotherapeutic and immunosuppressant agents might be effective in the treatment of asthma.[8] Low-dose weekly methotrexate is not without hazard. Both hepatotoxicity and pulmonary fibrosis have been reported in patients receiving similar therapy for psoriasis and rheumatoid arthritis.[8]

Gold Therapy

Because of its anti-inflammatory properties, gold therapy has been used as a standard therapy in Japan for the past 50 years.[9] However, these studies have been poorly controlled and better well-controlled trials are essential prior to subjecting patients to the potential serious toxicities associated with gold therapy. Similar to the findings with methotrexate, early studies with gold suggest its usefulness in the treatment of steroid-requiring asthma. Early trials with injectable gold demonstrated improvement in asthmatic symptoms, reduction in glucocorticoid requirements, and diminished airways reactivity.[9] A long-term open trial with oral gold (auranofin, Smith, Kline, and French) conducted in a similar population of severe asthmatics demonstrated a significant reduction in glucocorticoid use and decreased airways reactivity in about half of the patients evaluated.[8] Gold appears to require months to show efficacy, suggesting that its action might be via attenuation of the inflammatory response, as in arthritis. The relatively slow and incremental response observed, however, may be related to the severity of the patients studied. At this time, large multicenter controlled studies are being conducted to determine efficacy.

Other Agents

The nonsteroidal anti-inflammatory agents will be dealt with in detail in Chapter 26. Most asthmatics are unaffected by these agents but up to 25% may have their asthma aggravated; however, a few (less than 1%) are benefitted by these agents. Currently, there is no method of predicting which patients may benefit.

The use of expectorants has not demonstrated to be beneficial in asthma, although mucolytic therapy to assist removal of impacted mucus plugs in a large bronchus has been lifesaving in a few instances. Adequate hydration is usually all that is required. In acute asthma, the large negative intrathoracic pressures coupled with mediator-induced capillary permeability may predispose to pulmonary edema that will worsen oxygenation so that excessive hydration should be avoided.[9]

Aerosol Therapy for Asthma

Aerosol delivery of drugs for asthma has the advantages of being site-specific thus enhancing the therapeutic ratio. In addition, inhalation of β_2 agonists provides more rapid bronchodilation than either parenteral or oral administration as well as a greater degree of protection against EIA and other challenges.[43] Inhalation of glucocorticoids appears to have a greater effect on bronchial hyperreactivity than systemic administration,[26] and finally two prophylactic agents, cromolyn and nedocromil, are only effective by inhalation.[25] Therefore, an understanding of aerosol drug delivery is essential to optimal asthma therapy. Table 24.10 lists the factors determining lung deposition of therapeutic aerosols. They are divided into device and patient factors.

The various devices used to generate therapeutic aerosols include jet nebulizers, ultrasonic nebulizer, MDIs, and dry powder inhalers (DPI). The single most important device factor determining the site of aerosol deposition is droplet size.[46,47] Devices for delivering therapeutic aerosols generate particles with aerodynamic diameters from 0.5 to 35 μm in diameter.[46] Particles greater than 10 μm deposit in the oropharynx, particles between 5 and 10 μm deposit in the trachea and large bronchi, particles 1 to 5 μm reach the lower airways, and particles smaller than 0.5 μm act as a gas and are exhaled. Respirable particles are deposited in the airway by three mechanisms: (1) inertial impaction, (2) gravitational sedimentation, and (3) Brownian diffusion.[47] The first two are the most important for therapeutic aerosols and are probably the only factors that can be manipulated by patients.

The most important patient factor determining aerosol deposition is inspiratory rate. High inspiratory flow rates increase the degree of deposition due to impaction of all sized particles thereby increasing deposition centrally (i.e., large airways) and decreasing peripheral deposition.

Table 24.10 Factors Determining Lung Deposition of Aerosols

Device	*Device factors*	*Patient factors*
Metered-dose inhaler (MDI)	Canister held inverted Formulation (solution versus micronized suspension) Actuator cleanliness Addition of large volume spacer	Rate of inhalation (slow inspiration) Breathholding Coordinating actuation and inhalation Shaking device
Jet nebulizer	Volume fill (4–6 mL) Gas flow rate (6–12 L/min) Open versus closed system Dead space volume Thumb activating valve	Inhalation rate (slow, deep inspiration) Breathholding Tapping nebulizer
Ultrasonic nebulizer	Volume fill	Inhalation rate (slow, deep inspiration) Breathholding Tapping nebulizer
Dry powder inhaler (DPI)	Actuator cleanliness	Inhalation rate (rapid, deep inspiration) Breathholding Tilting head back

Besides the two major factors, there are a number of other factors that can be altered to improve delivery and efficacy of clinical aerosols. Most of these factors tend to be device specific and will be discussed under the individual device. Patient factors that cannot be controlled include intrapatient variability in airway geometry (particularly the differences between children and adults), the effect of bronchospasm edema, and mucus hypersecretion. Studies indicate that mild obstruction actually increases aerosol deposition;[23] however, severe obstruction probably leads to increased central deposition from impaction.[47]

Metered-Dose Inhalers Metered-dose inhalers are the most popular form of aerosol delivery due to their convenience (easy portability) and efficacy. They consist of a pressurized canister with a metering valve containing active drug, low vapor pressure propellants (i.e., chlorofluorocarbons), cosolvents, and/or surfactants.[23,25] The drug is either in solution or a suspended micronized powder. In order to disperse the suspension for accurate delivery, the canister must be shaken. The metering chamber measures a liquid volume and therefore the device must be held with the valve stem downward so that the chamber is covered with liquid.[23,25] The canister is placed inverted in an actuator and when pressed, the device releases the propellant and drug in a forceful spray whose particles are large [mass median aerodynamic diameter (MMAD) 45 μm] with an initial velocity of 100 mph.[23] As evaporation occurs, the particle size is reduced to a final MMAD of 2.8 to 5.5 μm, depending on the MDI. The aerosol cloud extends at least 10 inches beyond the MDI at the lowest MMAD.[25] Although chlorofluorocarbons can produce cardiac arrhythmias at high doses, investigations have failed to detect adverse effects from the dose delivered via MDIs in recommended dosages. Surfactants, particularly oleates, can produce lung irritation and coughing at excessive doses.[23,25]

Appropriate technique is required to achieve optimal drug delivery and therapeutic effect from an MDI (Table 24.10). Even with optimal technique, only about 10% (5% to 15%) of the metered dose is deposited in the lung. Approximately 80% impacts on the oropharynx due to the initial high velocity and this portion is then swallowed; the rest is either left in the actuator or exhaled.[47] It is important that actuation occurs during inhalation although the time during inspiration is unimportant provided the inspiratory rate is slow (30 L/min or 5 to 10 seconds for entire inspiration).[47] A number of authors advocate holding the actuator 2 to 3 cm in front of an open mouth to allow more evaporation and less impaction. Although radiolabel studies indicate improved delivery, physiologic studies with bronchodilators have failed to document an advantage for this method.[31] A large number of studies have shown that many patients do not use their MDIs optimally and also that patient instruction with demonstration is the most effective means of improving inhaler technique.[23,25,46,47] Even with instruction, up to 30% of patients, particularly young children and the elderly, cannot master the use of an MDI. For these patients the attachment of auxiliary devices or spacers to the MDI can significantly improve efficacy.[46,47]

Spacers Advantages to the use of spacers with an MDI are decreased oropharyngeal deposition and enhanced lung delivery.[47,48] However, not all spacer devices produce similar effects. The design of spacers varies from simple open-ended tubes that maintain the MDI away from the mouth to holding chambers with one-way valves that open during inhalation (Table 24.11). The purpose of a spacer is to allow evaporation of the propellant prior to inhalation. This allows inhalation after actuation of the device obviating the need for good hand-lung coordination and for a greater number of drug particles to achieve a respirable droplet size.[47] Additionally, most of the large particles that would normally deposit in the oropharynx rain out in the spacer.[25,47] All of the available spacers significantly reduce oropharyngeal deposition of aerosols from various MDIs.[48] This is an important factor in reducing local adverse effects (hoarseness, thrush) and may decrease HPA-axis suppression from inhaled glucocorticoids,[42,49] but has no clinical importance for bronchodilators delivered by MDI.[47] The use of spacers significantly enhances the clinical effect from bronchodila-

Table 24.11 Spacer Devices for Metered-Dose Inhalers

Aerochamber (Monaghan Medical Co.)	Holding chamber. Cylinder with one-way valve that releases aerosol when subject inhales with a flow indicator whistle. Also optional infant face mask attachment
Brethancer (Geigy Pharmaceuticals)	Tube spacer. A 10-cm, open-ended, telescopic plastic tube
Inhal-Aid (Key Pharmaceuticals)	Clear plastic holding chamber with flow meter and one-way valves for directed airflow
InspirEase (Key Pharmaceuticals)	Holding chamber consisting of a collapsible bag with a flow indicator whistle
Nebuhaler (AB Draco, Lund, Sweden)	750-mL conical holding chamber with one-way valve at the mouthpiece

tors in those patients with poor hand–lung coordination but offers no advantage in those patients who can optimally use an MDI alone, despite the fact that radiolabeled studies show an increased lung deposition.[46,47] Either the increased amount of bronchodilator drug deposited in the lung is clinically insignificant or the inconsistent result is due to the utilization of different spacers. Only the large volume spacers such as the Nebuhaler and InspirEase consistently increase aerosol lung delivery.[48] Thus the increased delivery appears to be clinically significant for inhaled glucocorticoids[42,49] but not bronchodilators.[47]

Jet Nebulizers Jet nebulizers are primarily used to deliver aerosols to hospitalized patients or patients with acute asthma exacerbations presenting to the clinic or emergency room. They have the advantage of not requiring significant patient coordination or cooperation other than tidal breathing. Jet nebulizers produce an aerosol from a liquid solution placed in a cup. A tube connected to a stream of compressed air or oxygen flows up through the bottom and draws the liquid up an adjacent open-ended tube. The air and liquid strike a baffle creating a droplet cloud that is then inhaled.[23,25] Large droplets adhere to the sides of the nebulizer and baffles, coalesce, and drip to the bottom to be renebulized. The aerosol output and lung delivery varies between the commercially available nebulizers even when operated in the same manner.[47] This is due to differing dead space volumes and baffle systems. Altering the operating parameters can also significantly effect lung delivery. Because dead space (i.e., the volume left behind after nebulization stops) remains constant, increasing fill volume will increase total amount of drug delivered; however, it will also take longer to nebulize the dose.[47] A total fill volume of 4 to 6 mL is considered optimal but will take 10 to 15 minutes to complete.[23,25] While this may be an inconvenience to the outpatient, the slower nebulization is probably an advantage in the patient experiencing an acute exacerbation. Tapping the side of the nebulizer during operation induces the droplets on the sides to fall back into the reservoir, minimizing loss.[46] The MMAD of the droplets is directly related to the gas flow rate with flow rates of 6 to 12 L/min, providing an aerosol cloud with MMAD of 4 to 8 μm for most nebulizers.[46] Putting a hole in the gas supply tube so nebulization will only occur during inhalation when the patients close their thumb over the hole also decreases aerosol loss. Quiet tidal breathing through a mouthpiece or face mask is the usual method of aerosol delivery from a nebulizer; however, slow deep inhalation and breathholding will also improve delivery from this device as well as from an MDI.

Approximately 10% (5% to 15%) of the dose placed in a nebulizer is delivered to the patient's lung with 60% to 80% lost in the apparatus, up to 20% exhaled, and 2% deposited in the mouth under usual operating conditions.[23,25,46,47] Ultrasonic nebulizers that produce an aerosol by vibrating liquid lying above a transducer at speeds of about 1 mHz produce similar degrees of lung deposition as jet nebulizers.[47] Thus, it is easy to see why patients not responding to multiple doses of bronchodilator via MDI during acute attacks respond to the usual doses administered via nebulizer. For example, 2.5 mg of albuterol via a nebulizer should deliver approximately 0.25 mg into the airways where 10 puffs from an MDI would only be expected to deliver 0.1 mg to the airways. This is without the increased risk of poor MDI technique during the attack. However, this should not be interpreted as meaning that jet nebulizers are superior to MDIs, for even in acute asthma when β_2 agonists are given in the same dosage by MDI plus spacer or nebulizer over the same time period, they have been shown to be equally effective.[50]

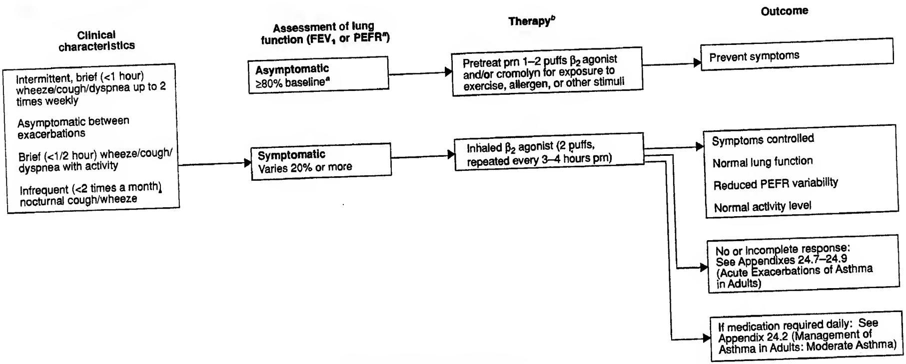
Dry Powder Inhalers (DPIs) Dry micronized powders can be inhaled directly into the lung. Cromolyn was first introduced in this fashion via a Spinhaler. Due to the proposed ban on the production of chlorofluorocarbon propellants, more companies are developing DPI devices. The currently available DPIs have the disadvantage that the dosage capsules must be carried separately and placed in the DPI prior to use; however, multidose DPIs are being developed and are in clinical studies.[25] The Spinhaler for cromolyn places a capsule on a propeller-driven spinning device, the capsule is punctured by two pins and the patient inhales. A capsule is placed in the back of the Rotahaler for albuterol, the device is twisted and the capsule breaks open releasing the medication on a mesh. The advantage of DPIs over MDIs is that they are breath activated so require little hand–lung coordination. However, they do require significantly higher inspiratory flow rates (80 to 120/min) to create respirable particles which in turn increase the degree of impaction.[23,47] Children under 5 years old are usually unable to generate adequate inspiratory flows. Up to 20% of a dose from a DPI can be deposited in the lung depending on the inspiratory flows, but in practice it is more usual to be around 10%; thus one capsule of albuterol (200 μg) is approximately equivalent to two puffs from an MDI. The capsules also contain lactose, which can produce irritation and coughing.

Summary

Asthma is a complicated disease with a multitude of clinical presentations. The exact defect in asthma has not been defined and it may be that asthma is a common presentation

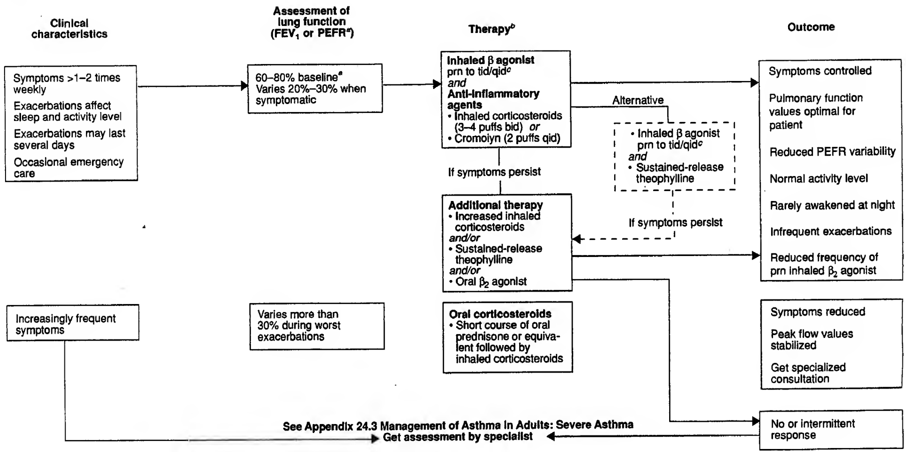
of a heterogenous group of diseases. Asthma is defined and characterized by excessive reactivity of the bronchial tree to a wide variety of noxious stimuli. The reaction is characterized by bronchospasm, excessive mucus production, and inflammation. The central role of inflammation in inducing and maintaining bronchial hyperreactivity is now becoming widely appreciated and studied. The goal of asthma therapy is to normalize, as much as possible, the patient's life and prevent chronic irreversible lung changes. Drugs are the mainstay of asthma therapy. The goal of drug therapy is to use the minimum amount possible to completely control the disease. In chronic asthma, therapy should be aimed at both bronchospasm and inflammation in order to produce the best results. Patients should be diligently followed and monitored for toxicities. Although death from asthma is an uncommon event, the most common cause of death is underassessment of the severity of obstruction either by the patient or clinician; the next common is undertreatment. A cornerstone of any therapy is education and the realization that most asthma deaths are avoidable.

Appendix 24.1 Management of Asthma in Adults: Mild Asthma



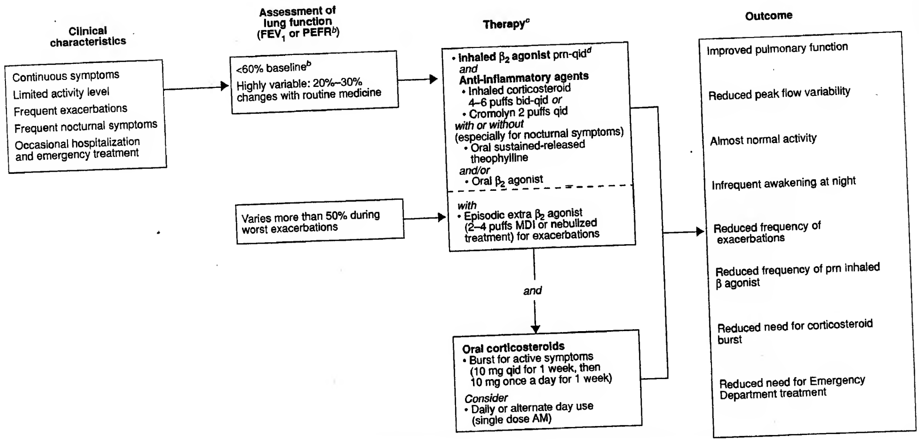
[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.
[b] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.

Appendix 24.2 Management of Asthma in Adults: Moderate Asthma



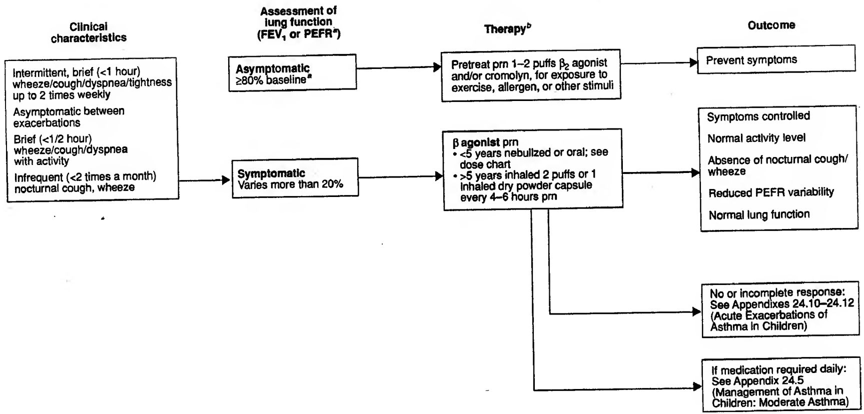
[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted based on standardized norms or % patient's personal best.
[b] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.
[c] If exceed 3–4 doses a day, additional therapy should be considered.

Appendix 24.3 Management of Asthma in Adults: Severe Asthma[a]



[a] Note: Individuals with severe asthma should be evaluated by an asthma specialist.
[b] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.
[c] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.
[d] If exceed 3–4 doses a day, additional therapy should be considered.

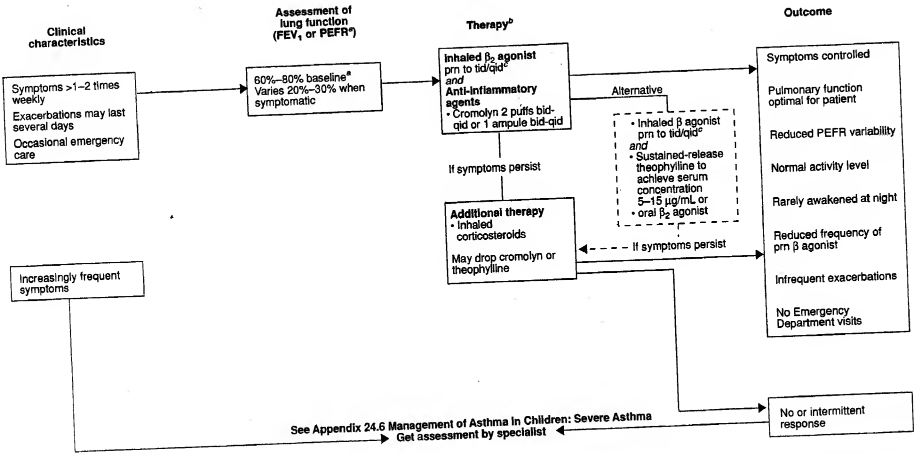
Appendix 24.4 Management of Asthma in Children: Mild Asthma



[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

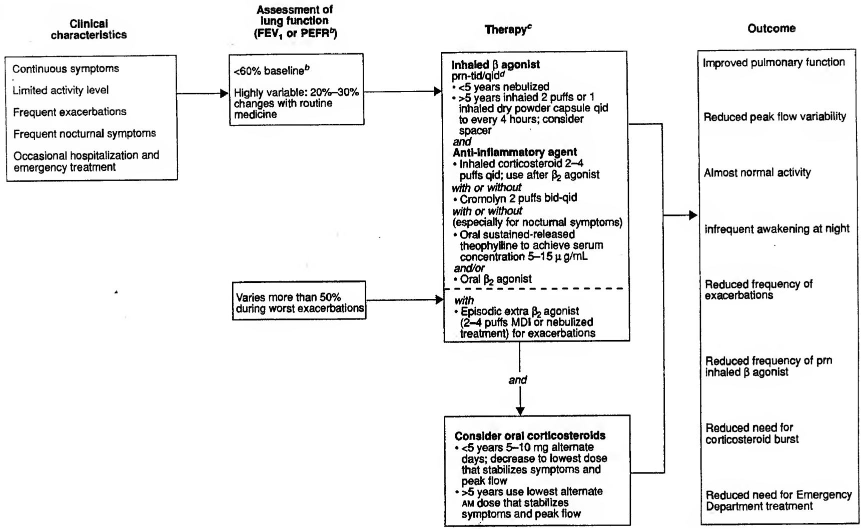
[b] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.

Appendix 24.5 Management of Asthma in Children: Moderate Asthma



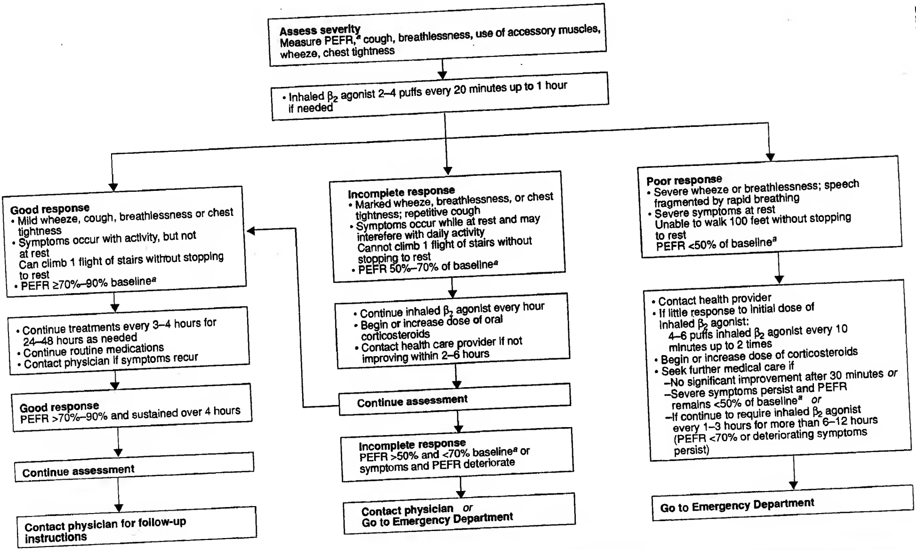
[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.
[b] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.
[c] If exceed 3–4 doses a day, additional therapy should be considered.

Appendix 24.6 Management of Asthma in Children: Severe Asthma[a]



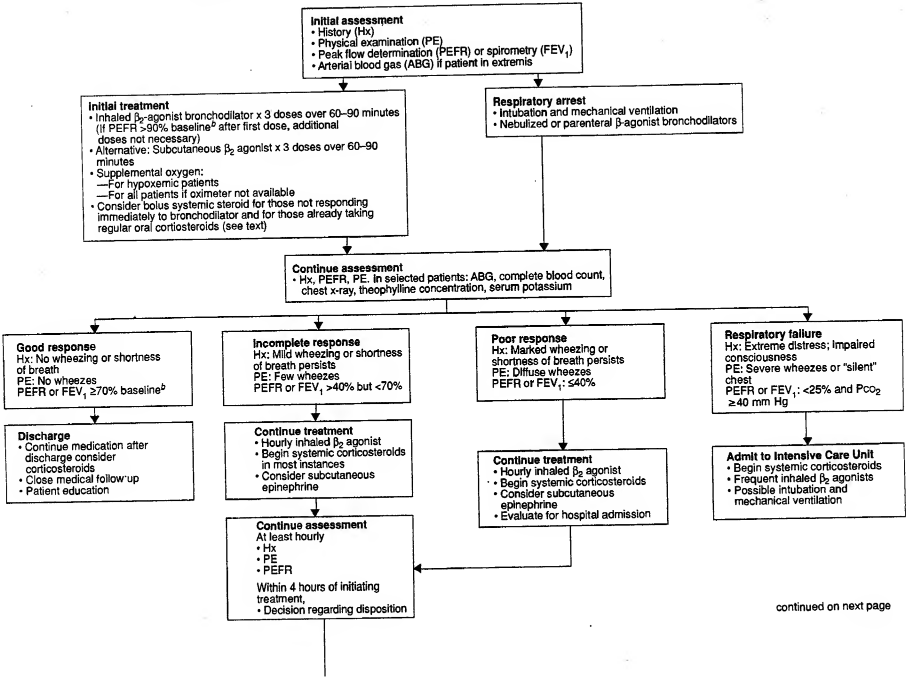
[a] Note: Individuals with severe asthma should be evaluated by an asthma specialist.
[b] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.
[c] All therapy must include patient education about prevention (including environmental control where appropriate) as well as control of symptoms.
[d] If exceed 3–4 doses a day, additional therapy should be considered.

Appendix 24.7 Acute Exacerbations of Asthma in Adults: Home Management



[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendix 24.8 Acute Exacerbations of Asthma in Adults: Emergency Department Management[a]



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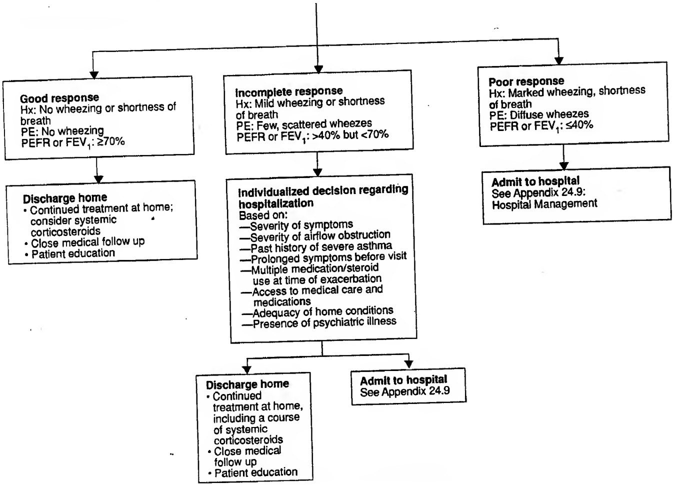
Appendix 24.8 Acute Exacerbations of Asthma in Adults: Emergency Department Management[a] (continued)



[a] Therapies are often available in a physician's office. However, most acutely severe exacerbations of asthma require a complete course of therapy in an Emergency Department.
[b] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendix 24.9 Acute Exacerbations of Asthma in Adults: Hospital Management

Initial assessment
- Detailed medical history (Hx)
- Complete physical examination (PE)
- Expiratory flow measurement: PEFR or FEV_1
- Chest radiograph
- Arterial blood gas/oximetry (see text)

Special attention for:
- Past history of respiratory failure
- Elderly
- Pregnant
- Complicating medical conditions
- History of steroid-induced complications (e.g., psychosis)

↓

Treatment
- Inhaled β_2 agonists up to every 1–2 hours
- Systemic corticosteroids: e.g., intravenous methylprednisolone 60–80 mg every 6–8 hours
- Intravenous aminophylline or oral theophylline
- Supplemental oxygen (if hypoxemic)

↓

Continued assessments
- Hx
- PE
- PEFR or FEV_1
  - Measurements before and after bronchodilator desirable
  - Measurements at least twice daily

↓

Improved
Suggested goals prior to discharge:
- Hx: No wheezing; good exercise tolerance: ≤1 awakening at night with mild asthmatic symptoms
- PE: Minimal or no wheezing
- PEFR or FEV_1: ≥70% of baseline[a]

↓

Preparation for discharge
- Inhaled β_2 agonist no more than every 3–4 hours
- Oral corticosteroids: role of inhaled corticosteroids discussed in text
- Oral theophylline
- Adequate oxygen saturation breathing room air
- Provide patient education, especially
  - Medication use
  - PEFR measurement at home
  - Need for follow up and chronic care (contact with physician within 7–10 days of discharge is recommended)

↓

Home with patient education, medications, and follow-up plan

Not Improved
Deterioration despite maximal therapy

Transfer ICU →

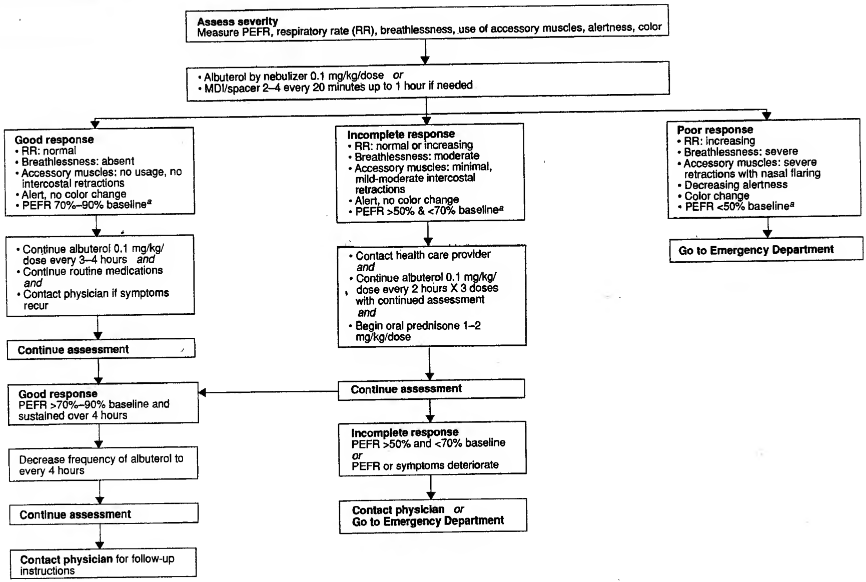
Intensive Care Unit (ICU)
- Pco_2 ≥40 mm Hg with severe airflow obstruction
- Deterioration despite maximal therapy

↓

ICU treatment
- Nebulized β_2 agonists every 30–60 minutes; may supplement with subcutaneous epinephrine
- Intravenous corticosteroids
- Intravenous aminophylline
- Oxygen supplementation
- Intubation and mechanical ventilation for hypercapnic respiratory failure

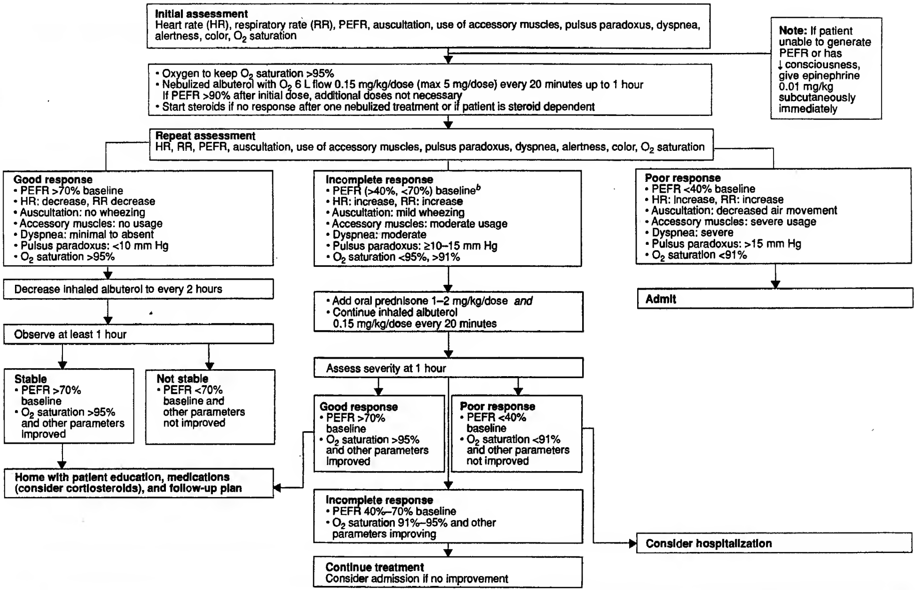
[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendix 24.10 Acute Exacerbations of Asthma in Children: Home Management



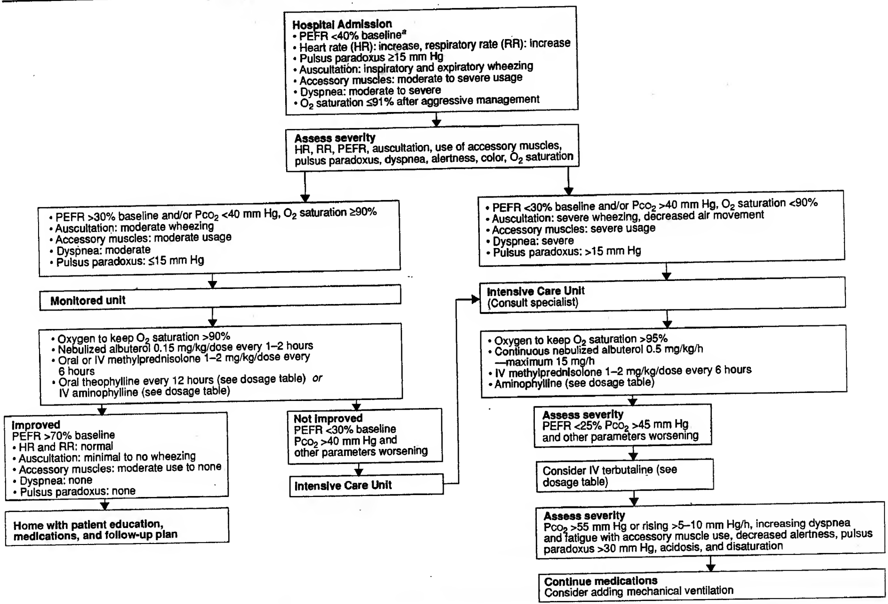
[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendix 24.11 Acute Exacerbations of Asthma in Children: Emergency Department Management[a]



[a] Therapies are often available in a physician's office. However, most acutely severe exacerbations of asthma require a complete course of therapy in an Emergency Department.
[b] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendix 24.12 Acute Exacerbations of Asthma in Children: Hospital Management



[a] PEFR % baseline refers to the norm for the individual, established by the clinician. This may be % predicted of standardized norms or % patient's personal best.

Appendixes from U.S. Department of Health and Human Services, Public Health Service. National Institutes of Health publication no. 913042.

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United States Patent [19]

Nyce

[11] Patent Number: 5,527,789

[45] Date of Patent: Jun. 18, 1996

[54] METHOD OF INHIBITING CARCINOGENESIS BY TREATMENT WITH DEHYDROEPIANDROSTERONE AND ANALOGS THEREOF

[75] Inventor: Jonathan W. Nyce, Greenville, N.C.

[73] Assignee: East Carolina University, Greenville, N.C.

[21] Appl. No.: 284,307

[22] Filed: Aug. 2, 1994

Related U.S. Application Data

[63] Continuation of Ser. No. 840,510, Feb. 24, 1992, abandoned.

[51] Int. Cl.[6] A61K 31/56; A61K 31/665; A61K 31/66; A61K 31/56; A61K 31/58

[52] U.S. Cl. 514/178; 514/99; 514/102; 514/121; 514/171; 514/172; 514/690

[58] Field of Search 514/178, 690, 514/99, 121, 102, 172, 171

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Primary Examiner—Jerome D. Goldberg

Attorney, Agent, or Firm—Bell, Seltzer, Park & Gibson

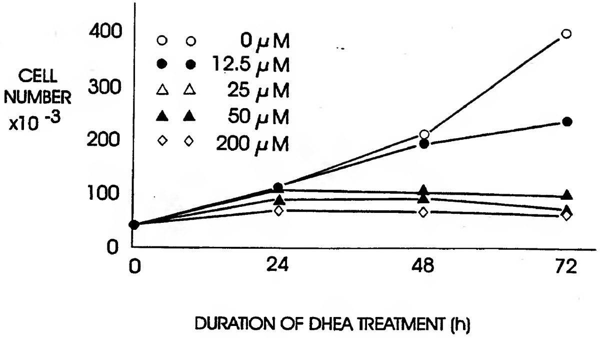
[57] **ABSTRACT**

A method of combatting cancer in a subject comprising administering to said subject dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer is disclosed in which heart failure induced by the DHEA or analog thereof is combatted by administering to the subject a ubiquinone, in an amount effective to combat heart failure induced by the DHEA or analog thereof.

A prefered DHEA analog for carrying out the invention is 16 alpha-fluoroepiandrosterone, and a preferred ubiquinone for carrying out the invention is Coenzyme Q_{10}.

19 Claims, 8 Drawing Sheets

FIG. 1


CELL NUMBER x10 -3
400
300
200
100
0
0
24
48
72
0 μM
12.5 μM
25 μM
50 μM
200 μM
DURATION OF DHEA TREATMENT (h)

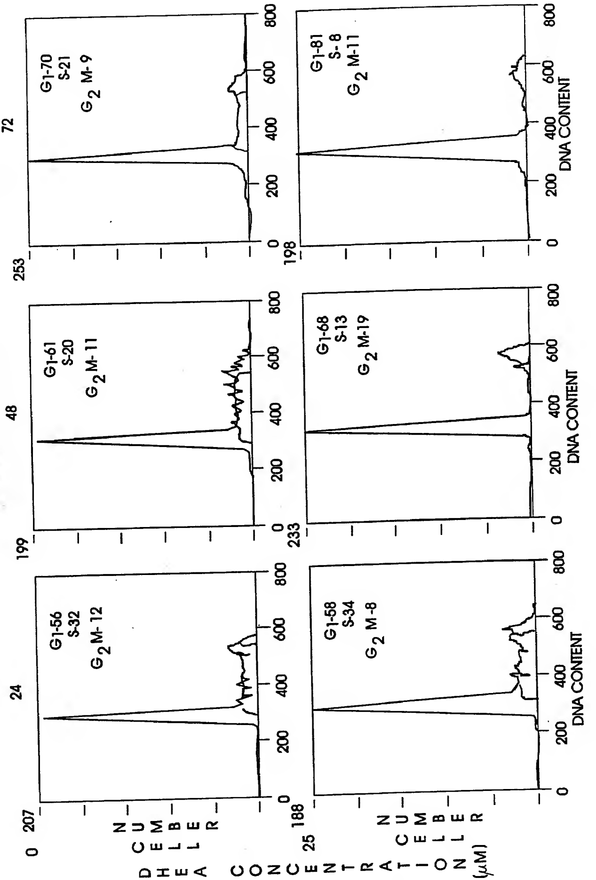

DURATION OF DHEA TREATMENT (h)
24
48
72
G1-56 S-32 G2 M-12
G1-61 S-20 G2 M-11
G1-70 S-21 G2 M-9
G1-58 S-34 G2 M-8
G1-68 S-13 G2 M-19
G1-81 S-8 G2 M-11
207
199
253
188
233
198
0
200
400
600
800
DNA CONTENT
NUMBER CELL
DHEA CONCENTRATION (μM)
0
25

FIG. 2A

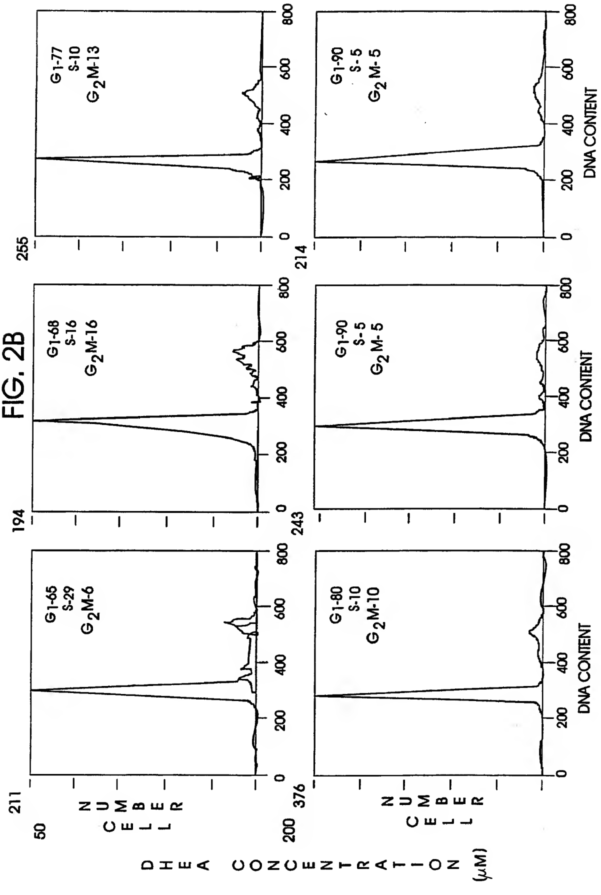

G1-77
S-10
G2M-13
G1-68
S-16
G2M-16
G1-65
S-29
G2M-6
G1-90
S-5
G2M-5
G1-90
S-5
G2M-5
G1-80
S-10
G2M-10
DNA CONTENT
CELL NUMBER
50
200
DHEA CONCENTRATION (μM)

FIG. 2B

FIG. 3A

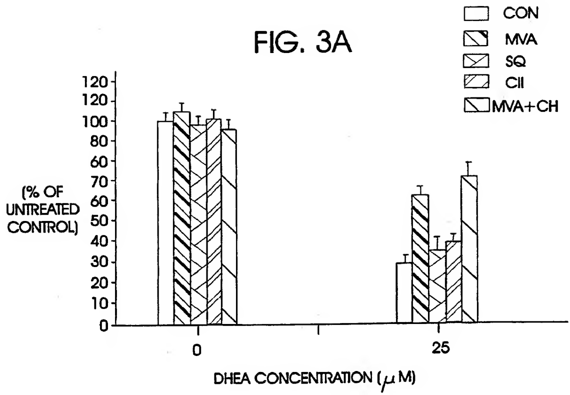
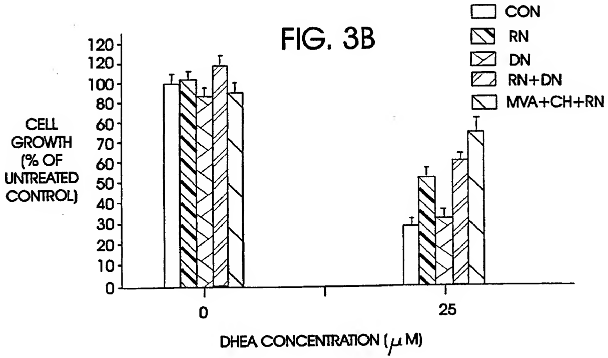

CON
MVA
SQ
CII
MVA+CH
120
120
100
80
60
70
60
50
40
30
20
10
0
(% OF UNTREATED CONTROL)
0
25
DHEA CONCENTRATION (μM)

FIG. 3B


CON
RN
DN
RN+DN
MVA+CH+RN
120
120
100
80
60
70
60
50
40
30
20
10
0
CELL GROWTH (% OF UNTREATED CONTROL)
0
25
DHEA CONCENTRATION (μM)

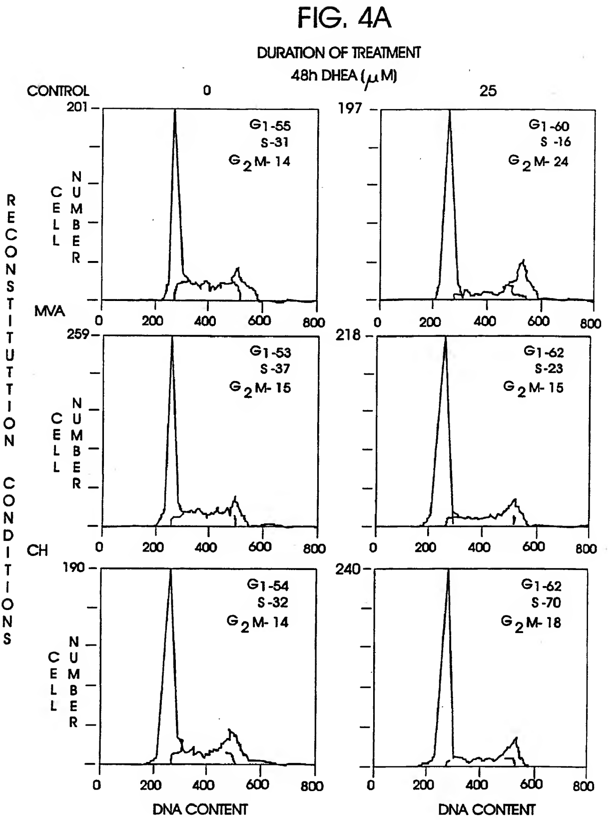


FIG. 4A
DURATION OF TREATMENT
48h DHEA (μM)
CONTROL
0
25
RECONSTITUTION CONDITIONS
MVA
CH
CELL NUMBER
DNA CONTENT
201
197
259
218
190
240
0
200
400
600
800
G1-55
S-31
G2M-14
G1-60
S-16
G2M-24
G1-53
S-37
G2M-15
G1-62
S-23
G2M-15
G1-54
S-32
G2M-14
G1-62
S-70
G2M-18

FIG. 4B

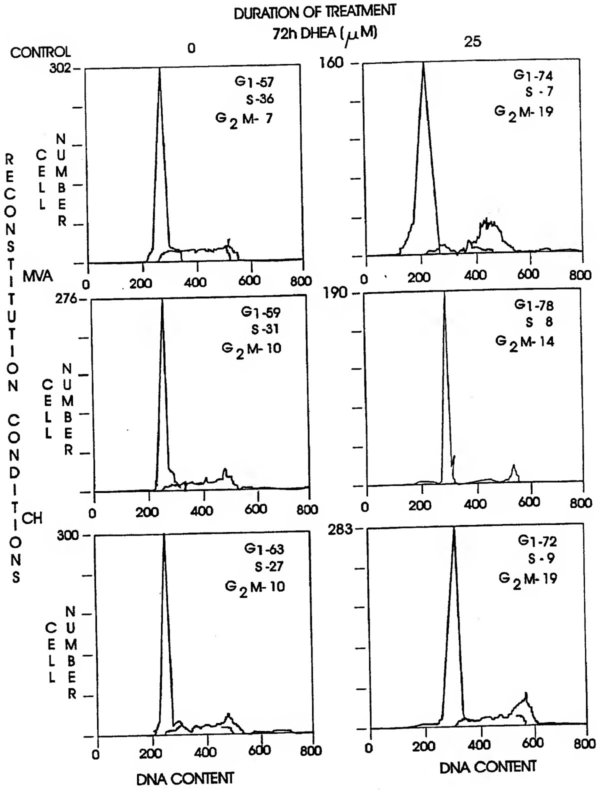

DURATION OF TREATMENT
72h DHEA (μM)
CONTROL
0
25
RECONSTITUTION CONDITIONS
MVA
CH
CELL NUMBER
DNA CONTENT
302
160
276
190
300
283
0
200
400
600
800
G1-57
S-36
G2M- 7
G1-74
S - 7
G2M- 19
G1-59
S-31
G2M- 10
G1-78
S 8
G2M- 14
G1-63
S-27
G2M- 10
G1-72
S - 9
G2M- 19

FIG. 4C

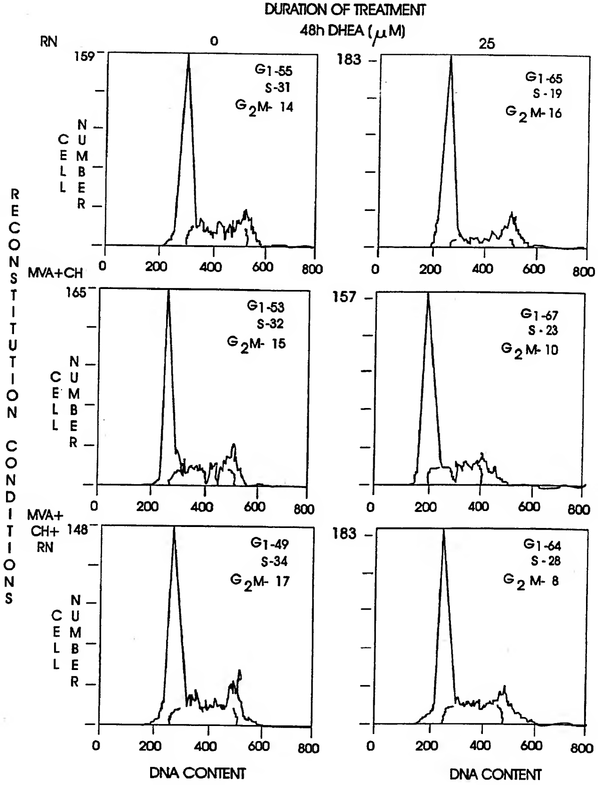
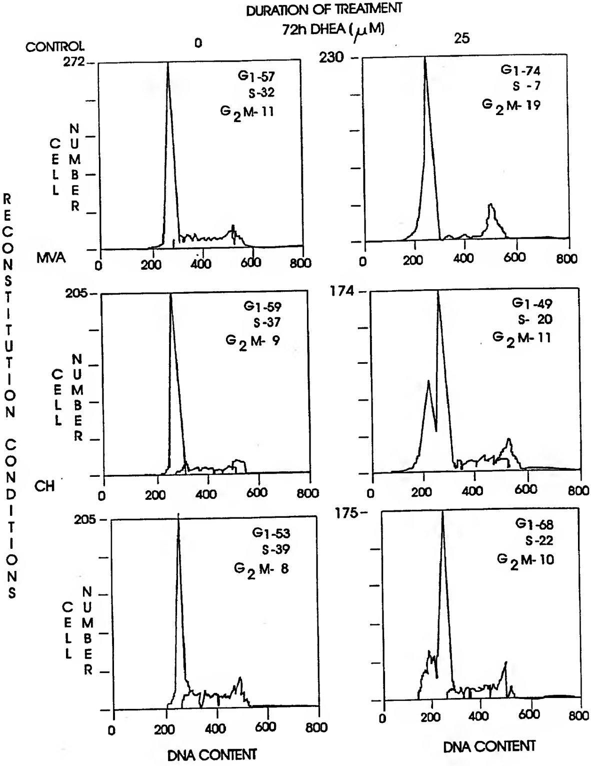

DURATION OF TREATMENT
48h DHEA (μM)
0
25
RECONSTITUTION CONDITIONS
RN
159
G1-55
S-31
G2M- 14
183
G1-65
S-19
G2M-16
MVA+CH
165
G1-53
S-32
G2M- 15
157
G1-67
S-23
G2M-10
MVA+ CH+ RN
148
G1-49
S-34
G2M- 17
183
G1-64
S-28
G2M- 8
CELL NUMBER
0
200
400
600
800
DNA CONTENT

FIG. 4D


DURATION OF TREATMENT
72h DHEA (μM)
0
25
RECONSTITUTION CONDITIONS
CONTROL
MVA
CH
CELL NUMBER
DNA CONTENT
272
230
205
174
205
175
0
200
400
600
800
G1-57
S-32
G2M-11
G1-74
S-7
G2M-19
G1-59
S-37
G2M-9
G1-49
S-20
G2M-11
G1-53
S-39
G2M-8
G1-68
S-22
G2M-10

METHOD OF INHIBITING CARCINOGENESIS BY TREATMENT WITH DEHYDROEPIANDROSTERONE AND ANALOGS THEREOF

This is a continuation of application Ser. No. 07/840,510 filed on 24 Feb. 1992, now abandoned.

FIELD OF THE INVENTION

The present invention relates to a method for combatting cancer by administering dehydroepiandrosterone (DHEA) or an analog thereof in combination with a ubiquinone, wherein the ubiquinone is administered in an amount effective to combat heart failure.

BACKGROUND OF THE INVENTION

DHEA is a naturally occurring steroid secreted by the adrenal cortex with apparent chemoprotective properties. Epidemiological research has shown that low endogenous levels of the natural steroid dehydroepiandrosterone (DHEA) correlate with increased risk of developing some forms of cancer, such as premenopausal breast cancer in women and bladder cancer in both sexes. R. D. Bulbrook et al., *Lancet* 2, 395–398 (1971); B. Zumoff, et al., *Cancer Res.* 41, 3360–3363 (1981); G. B. Gordon, *Cancer Res.* 51, 1366–1369 (1991); K. J. Helzlsouer, *Cancer. Res.* 52, 1–5 (1992). The ability of DHEA and DHEA analogs to inhibit carcinogenesis is believed to result from their uncompetitive inhibition of the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PDH).

G6PHD is the rate limiting enzyme of the hexose monophosphate pathway, a major source of intracellular ribose-5-phosphate and NADPH. P. A. Marks et al., *Proc. Nat. Acad. Sci. USA* 46, 447–452 (1960). Ribose-5-phosphate is a necessary substrate for the synthesis of both ribo- and deoxyribonucleotides required for the synthesis of RNA and DNA. NADPH is a cofactor also involved in nucleic acid biosynthesis and the synthesis of hydroxmethylglutaryl Coenzyme A reductase (HMG CoA reductase). S. Schulz et al., Inhibition of Protein Isoprenylation and p21ras Membrane Association by DHEA in Human Colonic Adenocarcinoma Cells in Vitro, *Cancer Res.* (Dec. 15, 1991).

HMG CoA reductase is a very unusual enzyme in that it requires two moles of NADPH for each mole of product, mevalonate, produced. Thus, it appears that HMG CoA reductase would be ultrasensitive to DHEA-mediated NADPH depletion, and that DHEA-treated cells would rapidly show depletion of intracellular pools of mevalonate. Mevalonate is required for DNA synthesis, and DHEA arrests human cells in the G1 phase of the cell cycle in a manner closely resembling that of the direct HMG CoA reductase inhibitor lovastatin. S. Schulz et al, Mechanism of Cell Growth Inhibition and Cell Cycle Arrest in Human Colonic Adenocarcinoma Cells by DHEA: Role of Isoprenoid Biosynthesis, Cancer Res. (submitted). Because G6PDH produces mevalonic acid used in cellular processes such as protein isoprenylation and the synthesis of dolichol (a precursor for glycoprotein biosynthesis), DHEA inhibits carcinogenesis by depleting mevalonic acid and thereby inhibiting protein isoprenylation and glycoprotein synthesis.

Mevalonate is the central precursor for the synthesis of cholesterol, as well as for the synthesis of a variety of nonsterol compounds involved in posttranslational modification of proteins (farnesyl pyrophosphate and geranlygeranyl pyrophosphate); for dolichol, which is required for the synthesis of glycoproteins involved in cell-to-cell communication and cell structure; and for ubiquinone, an antioxidant with an established role in cellular respiration. P. Mitchell, *Annals of the N.Y. Acad. Sci.* 341, 564 (1980); M. Gutman, *Biochem. Biophys. Acta.* 594, 53 (1980).

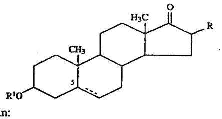
Adequate ubiquinone is essential for maintaining proper cardiac function and the addition of exogenous ubiquinone has recently been shown to have beneficial effect in patients with chronic heart failure. S. Greenberg et al., *J. Clin. Pharmacol.* 30, 596–608 (1990); S. A. Mortensen et al., *Int. J. Tiss. Reac.* 12(3), 155–162 (1990). Additionally, ubiquinone has been shown to be depleted in humans and animals treated with the direct HMG CoA reductase inhibitor lovastatin. K. Folkers et al., *Proc. Nat. Acad. Sci. USA* 87, 8931–8934 (1990); R. A. Willis et al., *Proc. Nat. Acad. Sci. USA* 87, 8928–8930 (1990). Such lovastatin-induced depletion of ubiquinone has been shown to lead to chronic heart failure (or to upgrading of low heart failure into life-threatening high grade heart failure). K. Folkers et al., *Proc. Nat. Acad. Sci. USA* 87, 8931–8934 (1990).

DHEA, unlike lovastatin, inhibits HMG CoA reductase indirectly by inhibiting G6PDH and depleting NADPH, a required cofactor for HMG CoA reductase. However, DHEA indirectly inhibits HMG CoA reductase sufficiently to deplete intracellular mevalonate. S. Schulz et al., Inhibition of Protein Isoprenylation and p21ras Membrane Association by DHEA in Human Colonic Adenocarcinoma Cells in Vitro, *Cancer Res.* (Dec. 15, 1991). This, too, will lead to ubiquinone depletion and consequent chronic heart failure following long term usage.

Thus although DHEA was once considered a safe drug, it is now predicted that with long term administration of DHEA or its analogs, chronic heart failure occurs as a complicating side effect. Further, some analogs of DHEA produce this side effect to a greater extent in that specific analogs have been reported to be a more potent inhibitor of G6PDH than DHEA.

SUMMARY OF THE INVENTION

A first aspect of the present invention is a method of combatting cancer, wherein dehydroepiandrosterone (DHEA) or an analog thereof is administered to the subject in an amount effective to combat cancer, and wherein a ubiquinone is administered to the subject in an amount effective to combat heart failure induced by the DHEA or analog thereof. In an embodiment of the invention, the dehydroepiandrosterone or analog thereof is represented by the formula:



(I)

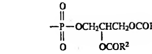
wherein:

R is hydrogen or a halogen; and

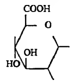
R^1 is hydrogen or an SO2OM group where M is hydrogen, sodium, a sulphatide group

$$-SO_2O-CH_2\underset{\displaystyle OCOR^2}{\underset{|}{C}H}CH_2OCOR^3$$

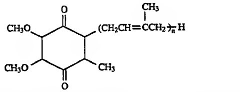
a phosphatide group



wherein each of R^2 and R^3, which may be the same of different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



and the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and the ubiquinone is represented by the formula:



(II)

wherein n=1 to 10. Preferably, R is a halogen, R^1 is hydrogen, and the double bond is present, and n is an integer from 6 to 10. More preferably the dehydroepiandrosterone or analog thereof is 16-alpha-fluoroepiandrosterone, and n is 10.

A second aspect of the present invention is a pharmaceutical formulation comprising DHEA or an analog thereof in an amount effective to combat cancer and a ubiquinone in an amount effective to combat heart failure together in a pharmaceutically acceptable carrier.

A third aspect of the present invention is the use of ubiquinone for the preparation of a medicament for combatting heart failure in a patient undergoing cancer-combatting treatment with DHEA or an analog thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings which form a portion of the disclosure of the invention:

FIG. 1 illustrates the inhibition of HT-29 SF cells by DHEA;

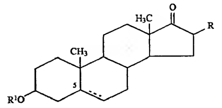
FIG. 2 illustrates the effects of DHEA on cell cycle distribution in HT-29 SF cells;

FIGS. 3A and 3B illustrate the reversal of DHEA-induced growth inhibition in HT-29 SF cells; and

FIG. 4 illustrates the reversal of DHEA-induced G_1 arrest in HT-29 SF cells.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, DHEA or an analog thereof is administered to a subject in an amount effective to combat cancer concurrently with a ubiquinone in an amount effective to combat heart failure. DHEA (dehydroisoandrosterone) is known (Merck Index Monograph No. 7710). Numerous DHEA analogs are also known. See, e.g., U.S. Pat. No. 4,956,355, UK Patent No. 2,240,472, EPO patent application Ser. No. 429,187 and PCT patent application Ser. No. 91/04030, the disclosures of which are to be incorporated herein by reference. Illustrative of DHEA and its analogs in accordance with the invention are compounds represented by the formula:

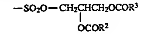


(I)

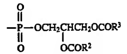
wherein:

R is hydrogen or a halogen (e.g., bromine, fluorine or chlorine);

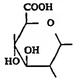
R^1 is hydrogen or an SO_2OM group where M is hydrogen, sodium, a sulphatide group



a phosphatide group



wherein each of R^2 and R^3, which may be the same of different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



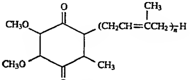
and wherein the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations.

Compounds illustrative of Formula (I) above include: DHEA wherein R and R^1 are each hydrogen and the double bond is present; 16 alpha-bromoepiandrosterone wherein R is Br, R^1 is H, and the double bond is present; 16 alpha-fluoroepiandrosterone wherein R is F, R^1 is H and the double bond is present; etiocholanolone wherein R and R^1 are each hydrogen and the double bond is absent; dehydroepiandrosterone sulphate wherein R is H, R^1 is SO_2OM, and M is Na, and the double bond is absent; and dehydroepiandrosterone sulphatide wherein R is H, R^1 is SO_2OM and M is a sulphatide group as defined above, and the double bond is absent. Preferably the DHEA or DHEA analog is a halogenated DHEA analog according to Formula I wherein R is Br, F, or Cl and R^1 is H and the double bond is present, and most preferably wherein R is F and R^1 is H and the double bond is present.

The compounds of Formula I are made in accordance with known procedures, or variations thereof, which will be apparent to those skilled in the art. See U.S. Pat. No. 4,956,355, UK Patent No. 2,240,472, EPO patent application Ser. No. 429,187 and PCT patent application Ser. No.

91/04030. See also M. Abou-Gharbia et al., *J. Pharm. Sci.* 70, 1154–1157 (1981), also incorporated herein by reference.

The ubiquinone compound is a structure based on a 2,3-dimethoxy-5-methylbenzoquinone nucleus with a variable terpenoid acid chain containing one to twelve mono-unsaturated trans-isoprenoid units. Such compounds are known in the art as "Coenzyme Q_n," in which n equals 1 to 12. These compounds are also known in the art as "ubiquinone(x)," in which x designates the total number of carbon atoms in the side chain and can be any multiple of 5. The ubiquinone compounds of the present invention are referred to herein as compounds represented by the formula:



(II)

wherein n=1 to 10. Preferably, in the method of the invention, the ubiquinone is a compound according to Formula II, wherein n=6 to 10 (e.g., Coenzymes Q_{6-10}), and most preferably n=10 (e.g., Coenzyme Q_{10}).

The phrase "concurrently administering," as used herein, means that DHEA or the DHEA analog and the ubiquinone are administered either (a) simultaneously in time (optionally by formulating the two together in a common carrier), or (b) at different times during the course of a common treatment schedule. In the latter case, the two compounds are administered at times sufficiently close for the ubiquinone to counterbalance the deterioration of the heart function resulting from the administration of DHEA or its analog.

Subjects to be treated by the method of the present invention include both human and animal (e.g., dog, cat, cow, horse) subjects, and are preferably mammalian subjects.

The active compounds (i.e., the ubiquinone and the DHEA or analog thereof) may be administered to the subject by any suitable means, such as orally, topically (including transdermally), or parenterally (e.g., by intraperitoneal, intraveneous, subcutaneous, or intramuscular injection), and in dosages known in the art. See, e.g., U.S. Pat. No. 4,956,355, UK Patent No. 2,240,472, EPO patent application Ser. No. 429,187, and PCT patent application Ser. No. 91/04030, which are incorporated by reference above. See also S. A. Mortensen et al., *Int. J. Tiss. Reac. XII*(3), 155–162 (1990), S. Greenberg et al., *J. Clin. Pharm.* 30, 596–608 (1990), and K. Folkers, et al., *Proc. Nat'l. Acad. Sci.* 87, 8931–8934 (1990), also incorporated herein by reference.

Note that the DHEA or analog thereof may or may not be administered for a time sufficient to deplete endogenous ubiquinone. If the DHEA or analog thereof is administered for a time sufficient to deplete endogenous ubiquinone, then the administration of exogenous ubiquinone replenishes the level of ubiquinone. If the DHEA or analog thereof is administered for a time sufficient to deplete endogenous ubiquinone, then the administration of exogenous ubiquinone offsets future depletion.

In general, the ubiquinone is administered in an amount effective to combat heart failure, and the dosage will vary depending upon the condition of the subject and the route of administration. The ubiquinone is preferably administered in a total amount per day of about 1 to 1200 mg/kg body weight, more preferably about 30 to 600 mg/kg, and most preferably about 50 to 150 mg/kg. The ubiquinone may be administered once or several times a day.

The DHEA or DHEA analog is, in general, administered in an amount effective to combat cancer, and the dosage will likewise vary depending upon the condition of the subject and the route of administration. The DHEA or DHEA analog is preferably administered in a total amount per day of about 1 to 3600 mg/kg body weight, more preferably about 5 to 1800 mg/kg, and most preferably about 20 to 100 mg/kg. The DHEA or DHEA analog may be administered once or several times a day.

The compounds of Formula I may be administered per se or in the form of a pharmaceutically acceptable salt. When used in medicine, the salts of the compounds of Formula (I) should be both pharmacologically and pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare the free active compound or pharmaceutically acceptable salts thereof and are not excluded from the scope of this invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluenesulfonic, tartaric, citric, methanesulphonic, formic, malonic, succinic, naphthalene-2-sulphonic and benzenesulphonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group. Thus, the present invention also provides pharmaceutical formulations, both for veterinary and for human medical use, which comprise the ubiquinone together with one or more pharmaceutically acceptable carriers thereof and optionally any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not unduly deleterious to the recipient thereof.

Pharmaceutical formulations of the present invention may optionally include DHEA or DHEA analogs, preferably as described above. Such a formulation is useful for concurrently administering DHEA or a DHEA analog and the ubiquinone in a method as described above.

The formulations include those suitable for oral, rectal, topical, transdermal, nasal, ophthalmic or parenteral (including subcutaneous, intramuscular and intravenous) administration. Formulations suitable for oral and parenteral administration are preferred.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the potentiating agent as a powder or granules; or a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, with the active compound being in a free-flowing form such as a powder or granules which is optionally mixed with a binder, disintegrant, lubricant, inert diluent, surface

active agent or dispersing agent. Molded tablets comprised of a mixture of the powdered active compound with a suitable carrier may be made by molding in a suitable machine.

A syrup may be made by adding the active compound to a concentrated aqueous solution of a sugar, for example sucrose to which may also be added any accessory ingredient(s). Such accessory ingredient(s) may include flavorings, suitable preservatives, an agent to retard crystallization of the sugar, and an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient.

Nasal spray formulations comprise purified aqueous solutions of the active compound with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes.

Formulations for rectal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids.

Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye.

Topical formulations comprise the active compound dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols or other bases used for topical pharmaceutical formulations. The addition of other accessory ingredients, vide infra, may be desirable.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, flavoring agents, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

Example 1

Preparation of the Experimental Model

Cell cultures. HT-29 SF cells, which represent a subline of HT-29 cells (ATCC, Rockville, Md.) and are adapted for growth in completely defined serum-free PC-1 medium (Ventrex, Portland, Me.), were obtained. Stock cultures were maintained in this medium at 37° C. in a humidified atmosphere containing 5% CO_2. At confluence cultures were replated after dissociation using trypsin/EDTA (Gibco, Grand Island, N.Y.) and re-fed every 24 hours. Under these conditions, the doubling time for HT-29 SF cells during logarithmic growth was 24 hours.

Flow Cytometry. Cells were plated at 10^5/60-mm dish in duplicate. For analysis of cell cycle distribution, cultures were exposed to either 0, 25, 50, or 200 μM DHEA. For analysis of reversal of cell cycle effects of DHEA, cultures were exposed to either 0 or 25 μM DHEA, and the media were supplemented with MVA, CH, RN, MVA plus CH, or MVA plus CH plus RN or were not supplemented. Cultures were trypsinized following 0, 24, 48, or 74 hours and fixed and stained using a modification of a procedure of Bauer et al., *Cancer Res.*, 46, 3173–3178 (1986). Briefly, cells were collected by centrifugation and resuspended in cold phosphate-buffered saline. Cells were fixed in 70% ethanol, washed, and resuspended in phosphate-buffered saline. One ml hypotonic stain solution [50 μg/ml propidium iodide (Sigma Chemical Co.), 20 μg/ml RNase A (Boehringer Mannheim, Indianapolis, Ind.), 30 mg/ml polyethylene glycol, 0.1% Triton X-100 in 5 mM citrate buffer] was then added, and after 10 min at room temperature, 1 ml of isotonic stain solution [propidium iodide, polyethylene glycol, Triton X-100 in 0.4M NaCl] was added and the cells were analyzed using a flow cytometer, equipped with pulse width/pulse area doublet discrimination (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). After calibration with fluorescent beads, a minimum of 2×10^4 cells/sample were analyzed, data were displayed as total number of cells in each of 1024 channels of increasing fluorescence intensity, and the resulting histogram was analyzed using the Cellfit analysis program (Becton Dickinson).

Example 2

Analysis of Growth Inhibition and Cell Cycle Arrest by DHEA

Growth Inhibition Assay. Cells were plated 25,000 cells/30-mm dish in quadruplicate, and after 2 days received 0, 12.5, 25, 50, or 200 μM DHEA. Cell number was determined 0, 24, 48, and 72 hours later using a Coulter counter (model Z_f; Coulter Electronics, Inc., Hialeah, Fla.). DHEA (AKZO, Basel, Switzerland) was dissolved in dimethyl sulfoxide, filter sterilized, and stored at −20° C. until use.

FIG. **1** illustrates the inhibition of growth for HT-29 cells by DHEA. Points refer to numbers of cells, and bars refer to SEM. Each data point was performed in quadruplicate, and the experiment was repeated three times. Where SEM bars are not apparent, SEM was smaller than symbol. Exposure to DHEA resulted in a reduced cell number compared to controls after 72 hours in 12.5 μM, 48 hours in 25 or 50 μM, and 24 hours in 200 μM DHEA, indicating that DHEA produced a time- and dose-dependent inhibition of growth.

Cell Cycle Arrest by DHEA. To examine the effects of DHEA on cell cycle distribution, HT-29 SF cells were plated (10^5 cells/60 mm dish), and 48 hours later treated with 0, 25, 50, or 200 μM DHEA. FIG. **2** illustrates the effects of DHEA on cell cycle distribution in HT-29 SF cells. After 24, 48, and 72 hours, cells were harvested, fixed in ethanol, and stained with propidium iodide, and the DNA content/cell was determined by flow cytometric analysis. The percentage of cells in G_1, S, and G_2M phases was calculated using the Cellfit cell cycle analysis program. S phase is marked by a quadrangle for clarity. Representative histograms from duplicate determinations are shown. The experiment was repeated three times.

The cell cycle distribution in cultures treated with 25 or 50 μM DHEA was unchanged after the initial 24 hours. However, as the time of exposure to DHEA increased, the proportion of cells in S phase progressively decreased, and the percentage of cells in G_1 phase was increased after 72 hours. A transient increase in G_2M phase cells was apparent after 48 hours. Exposure to 200 μM DHEA produced a similar but more rapid increase in the percentage of cells in G_1 and a decreased proportion of cells in S phase after 24 hours, which continued through the treatment. This indicates that DHEA produced a G_1 block in HT-29 SF cells in a time- and dose-dependent manner.

Example 3

Analysis of Reversal of DHEA-mediated Growth Inhibition and Reversal of DHEA-induced Cell Cycle Arrest

Reversal of DHEA-mediated Growth Inhibition. Cells were plated as above, and after 2 days received either 0 or 25 μM DHEA-containing medium supplemented with mevalonic acid ("MVA"; 2 mM), squalene ("SQ"; 80 μM), cholesterol ("CH"; 15 μg/ml), MVA plus CH, ribonucleosides ("RN"; uridine, cytidine, adenosine, and guanosine at final concentrations of 30 μM each), deoxyribonucleosides ("DN"; thymidine, deoxycytidine, deoxyadenosine and deoxyguanosine at final concentrations of 20 μM each), RN plus DN, or MVA plus CH plus RN, or medium that was not supplemented. All compounds were obtained from Sigma Chemical Co. (St. Louis, Mo.). Cholesterol was solubilized in ethanol immediately before use. RN and DN were used in maximal concentrations shown to have no effects on growth in the absence of DHEA.

FIG. 3 illustrates the reversal of DHEA-induced growth inhibition in HT-29 SF cells. In A, the medium was supplemented with 2 μM MVA, 80 μM SQ, 15 μg/ml CH, or MVA plus CH (MVA+CH) or was not supplemented (CON). In B, the medium was supplemented with a mixture of RN containing uridine, cytidine, adenosine, and guanosine in final concentrations of 30 μM each; a mixture of DN containing thymidine, deoxycytidine, deoxyadenosine and deoxyguanosine in final concentrations of 20 μM each; RN plus DN (RN+DN); or MVA plus CH plus RN (MVA+CH+RN). Cell numbers were assessed before and after 48 hours of treatment, and culture growth was calculated as the increase in cell number during the 48 hour treatment period. Columns represent cell growth percentage of untreated controls; bars represent SEM. Increase in cell number in untreated controls was 173,370±6518. Each data point represents quadruplicate dishes from four independent experiments. Statistical analysis was performed using Student's t test. *, $P<0.01$; **, P, 0.001; compared to treated controls. Note that supplements had little effect on culture growth in absence of DHEA.

Under these conditions, the DHEA-induced growth inhibition was partially overcome by addition of MVA as well as by addition of MVA plus CH. Addition of SQ or CH alone had no such effect. This suggests that the cytostatic activity of DHEA was in part mediated by depletion of endogenous mevalonate and subsequent inhibition of the biosynthesis of an early intermediate in the cholesterol pathway that is essential for cell growth. Furthermore, partial reconstitution of growth was found after addition of RN as well as after addition of RN plus DN but not after addition of DN, indicating that depletion of both mevalonate and nucleotide pools is involved in the growth-inhibitory action of DHEA. However, none of the reconstitution conditions including the combined addition of MVA, CH, and RN completely overcame the inhibitory action of DHEA, suggesting either cytotoxic effects or possibly that additional biochemical pathways are involved.

Reversal of DHEA-induced Cell Cycle Arrest. HT-29 SF cells were treated with 25 μM DHEA in combination with a number of compounds, including MVA, CH, or RN, to test their ability to prevent the cell cycle-specific effects of DHEA. Cell cycle distribution was determined after 48 and 72 hours using flow cytometry.

FIG. 4 illustrates reversal of DHEA-induced arrest in HT-29 SF cells. Cells were plated (10^5 cells/60 mm dish) and 48 hours later treated with either 0 or 25 μM DHEA. The medium was supplemented with 2 μM MVA; 15 μg/ml CH; a mixture of RN containing uridine, cytidine, adenosine, and guanosine in final concentrations of 30 μM; MVA plus CH (MVA+CH); or MVA plus CH plus RN (MVA+CH+RN) or was not supplemented. Cells were harvested after 48 or 72 hours, fixed in ethanol, and stained with propidium iodine, and the DNA content per cell was determined by flow cytometric analysis. The percentage of cells in G_1, S, and G_2M phases were calculated using the Cellfit cell cycle profile analysis program. S phase is marked by a quadrangle for clarity. Representative histograms from duplicative determinations are shown. The experiment was repeated two times. Note that supplements had little effect on cell cycle progression in the absence of DHEA.

With increasing exposure time, DHEA progressively reduced the proportion of cells in S phase. While inclusion of MVA partially prevented this effect in the initial 48 hours but not after 72 hours, the addition of MVA plus CH was also able to partially prevent S phase depletion at 72 hours, suggesting a requirement of both MVA and CH for cell progression during prolonged exposure. The addition of MVA, CH, and RN was apparently most effective at reconstitution but still did not restore the percentage of S phase cells to the value seen in untreated control cultures. CH or RN alone had very little effect at 48 hours and no effect at 72 hours. Morphologically, cells responded to DHEA by acquiring a rounded shape, which was prevented only by the addition of MVA to the culture medium (data not shown). Some of the DNA histograms after 72 hours DHEA exposure in FIG. 4 also show the presence of a subpopulation of cells possessing apparently reduced DNA content. Since the HT-29 cell line is known to carry populations of cells containing varying numbers of chromosomes (68–72; ATCC), this may represent a subset of cells that have segregated carrying fewer chromosomes.

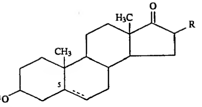
The examples above provide evidence that in vitro exposure of HT-29 SF human colonic adenocarcinoma cells to concentrations of DHEA known to deplete endogenous mevalonate results in growth inhibition and G_1 arrest and that addition of MVA to the culture medium in part prevents these effects. DHEA produced effects upon protein isoprenylation which were in many respects similar to those observed for specific 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors such as lovastatin and compactin. Unlike direct inhibitors of mevalonate biosynthesis, however, DHEA mediates its effects upon cell cycle progression and cell growth in a pleiotropic manner involving ribo- and deoxyribonucleotide biosynthesis and possibly other factors as well.

The foregoing examples are illustrative of the present invention, and are not to be taken as restrictive thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

That which is claimed is:

1. In a method of combatting cancer in a subject comprising administering to said subject dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer, wherein said cancer is sensitive to said DHEA or analog thereof, the improvement comprising administering to said subject a ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof,

wherein said DHEA or analog thereof is represented by the formula:

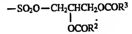


(I)

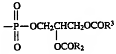
wherein:

R is hydrogen or a halogen;

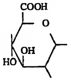
R^1 is hydrogen or an SO_2OM group where M is hydrogen, sodium, a sulphatide group



a phosphatide group

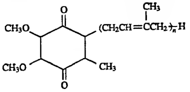


wherein each of R^2 and R^3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



and the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and

said ubiquinone represented by the formula:



(II)

wherein n=1 to 10.

2. The method according to claim 1 wherein said dehydroepiandrosterone or analog thereof is represented by Formula (I) wherein R is bromine, fluorine or chlorine, R^1 is hydrogen, and the double bond is present.

3. The method according to claim 1 wherein said dehydroepiandrosterone or analog thereof is 16-alpha-fluoroepiandrosterone.

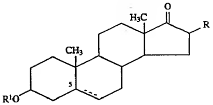
4. The method according to claim 1 wherein n is an integer from 6 to 10.

5. The method according to claim 1 wherein n is 10.

6. A method according to claim 1, wherein said dehydroepiandrosterone or analog thereof is administered to said subject parenterally and said ubiquinone is administered to said subject parenterally.

7. A method of combatting cancer in a subject comprising administering to said subject dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer, wherein said cancer is sensitive to said DHEA or analog thereof, concurrently with a ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof,

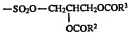
said DHEA or analog thereof represented by the formula:



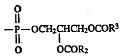
wherein:

R is hydrogen or a halogen;

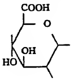
R^1 is hydrogen or an SO_2OM group where M is hydrogen, sodium, a sulphatide group



a phosphatide group

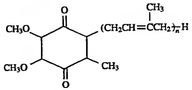


wherein each of R^2 and R^3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



and the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and

said ubiquinone represented by the formula:

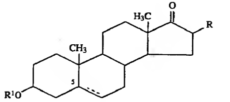


(II)

wherein n=1 to 10.

8. A method of combatting cancer in a subject comprising concurrently administering to said subject dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer, wherein said cancer is sensitive to said DHEA or analog thereof, with a ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof,

said DHEA or analog thereof represented by the formula:



(I)

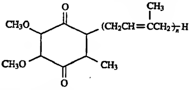
wherein

R is a halogen;

R^1 is hydrogen; and

the broken line represents an optional double bond and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and

said ubiquinone represented by the formula:

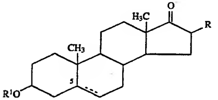


(II)

wherein n=6 to 10.

9. The method according to claim **8**, wherein said DHEA or analog thereof is 16-alpha-fluoroepiandrosterone and wherein n=10.

10. In a method of combatting cancer in a subject comprising administering to said subject dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer, wherein said cancer is sensitive to said DHEA or analog thereof, the improvement comprising administering to said subject an ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof, wherein said DHEA or analog thereof is represented by the formula:



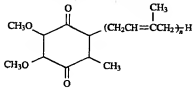
(I)

wherein

R is bromine, fluorine or chlorine;

R^1 is hydrogen; and

the double bond is present; and wherein said ubiquinone is represented by the formula:

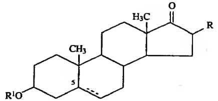


(II)

wherein n=10.

11. In a method comprising administering to a subject dehydroepiandrosterone (DHEA) or an analog thereof in need of such treatment in a therapeutically effective amount, the improvement comprising administering to said subject a ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof,

wherein said DHEA or analog thereof is represented by the formula:

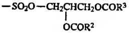


(I)

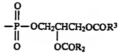
wherein:

R is hydrogen or a halogen;

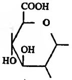
R^1 is hydrogen or an SO_2OM group where M is hydrogen, sodium, a sulphatide group



a phosphatide group

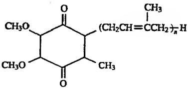


wherein each of R^2 and R^3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



and the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and

said ubiquinone represented by the formula:



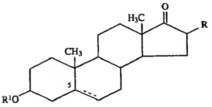
(II)

wherein n=1 to 10.

12. A method according to claim **11**, wherein R is bromine, fluorine, or chlorine; R^1 is hydrogen; and the double bond is present; and wherein n=10.

13. A pharmaceutical formulation comprising dehydroepiandrosterone (DHEA) or an analog thereof in an amount effective to combat cancer, wherein said cancer is sensitive to said DHEA or analog thereof, and a ubiquinone in an amount effective to combat heart failure induced by said DHEA or analog thereof in a pharmaceutically acceptable carrier,

wherein said DHEA or analog thereof is represented by the formula:

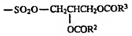


(I)

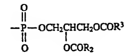
wherein:

R is hydrogen or a halogen;

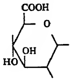
R^1 is hydrogen or an SO_2OM group where M is hydrogen, sodium, a sulphatide group



a phosphatide group

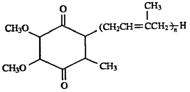


wherein each of R^2 and R^3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



and wherein the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the alpha or beta configuration or the compound comprises a mixture of both configurations; and

said ubiquinone represented by the formula:



(II)

wherein n=1 to 10.

14. A pharmaceutical formulation according to claim **11** wherein said DHEA or analog thereof is represented by Formula I, wherein R is bromine, fluorine, or chlorine, and R^1 is hydrogen, and the double bond is present.

15. A pharmaceutical formulation according to claim **11** wherein said DHEA or analog thereof is 16 alpha-fluoroepiandrosterone.

16. A pharmaceutical formulation according to claim **11** wherein said ubiquinone is represented by Formula II, wherein n is an integer from 6 to 10.

17. A pharmaceutical formulation according to claim **11** wherein said ubiquinone is represented by Formula II, wherein n is 10.

18. A pharmaceutical formulation according to claim **12** wherein said pharmaceutically acceptable carrier is an aqueous carrier.

19. A pharmaceutical formulation according to claim **13** wherein said pharmaceutically acceptable carrier is a solid carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,527,789

DATED : June 18, 1996

INVENTOR(S) : Jonathan W. Nyce

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 1, after line 7, please insert the following new paragraph:

--This invention was made with Government support under Grant No. CA47217, awarded by the National Cancer Institute. The Government has certain rights in the invention.--

In column 2, line 61, "SO2OM" should be --SO_2OM--.

Signed and Sealed this

Twenty-fourth Day of June, 1997

Attest:

Bruce Lehman

Mary A. Green

Attesting Officer

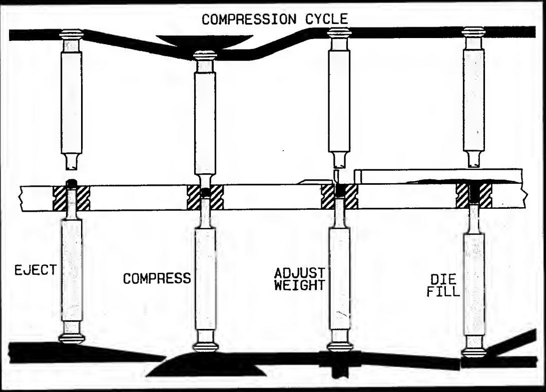
BRUCE LEHMAN

Commissioner of Patents and Trademarks

Pharmaceutical Dosage Forms: Tablets Volume 2

Second Edition, Revised and Expanded

Edited by Herbert A. Lieberman, Leon Lachman, and Joseph B. Schwartz



PHARMACEUTICAL DOSAGE FORMS

Tablets

SECOND EDITION, REVISED AND EXPANDED

In Three Volumes
VOLUME 2

EDITED BY

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population being samples in the enclosure (drum, mixer, storage hopper, etc.).

Probably the most significant measure of quality of a mixture is how the blend actually performs, and the uniformity of the final product. However, Current Good Manufacturing Practice (CGMP) Regulations require documentation of a controlled process at each step of manufacturing.

V. MATERIAL PROPERTIES: BASIC CONCEPTS OF DRY BLENDING—THE UNIT PARTICLE

Since mixing plays such an important role in tableting, an understanding of the characteristics of the materials being mixed is paramount. Many of the studies presented in the literature, and used previously in examples, deal with binary mixtures of physically and chemically similar materials which can easily be differentiated for the study by color, size, or assay. However, pharmaceutical, binary, particulate systems in tableting are the exception, and results dealing with binary systems have limited applicability in industrial practice.

Each component in a mixture has distinct physical characteristics which contribute to, or detract from, the completeness (uniformity) of a mixture. Therefore, it is important to define and characterize the unit particles that make up the mixture, whether it is a premix of a wet granulation, a direct compression formula, or the addition of lubricants, etc., to a granulation. Figure 21 is an illustration of several different types of particles handled in tablet granulation mixing.

The unit particles in a system may range from the less-than-1 μm-size pure substance raw material particle to the 8 to 12 mesh multicomponent granule held together by a binder. Since dry mixing is a dynamic state of an assemblage of particles, the properties of the unit particle must be discussed in terms affecting these dynamics.

There are three properties intrinsic to each component in the mixture: "composition (physicochemical structure), size (and size distribution), and shape" [31].

Composition of each particle is "its qualitative and quantitative makeup" [32]. Each unit of pure substance has its own molecular composition and arrangement that distinguishes it from all other materials, and dictates its behavior in part as a powder per se, or in combination with other tablet mixture ingredients. Chemical composition is important, because chemical reactivity limits a material's use with other tableting components, e.g., acids and bases such as aspirin and phenylpropanolamine would not be blended together because of their potential to react. The same applies to components that may affect the stability of a mixture such as the potential Schiff Base reaction between certain sugars and amines when in contact even in the dry state.

Physically, the molecular makeup determines crystallinity manifested as color, hardness, tackiness, general appearance, etc.

Particle size and size distribution of the unit particles have considerable impact on the flow properties of powders and therefore, the dynamics of mixing. Table 5 shows, in general, the effect of particle size on the flow properties of powders. Table 6 is a list of some common substances used in the pharmaceutical industry, and their flow characteristics. A very complete and detailed list of materials and their characteristics

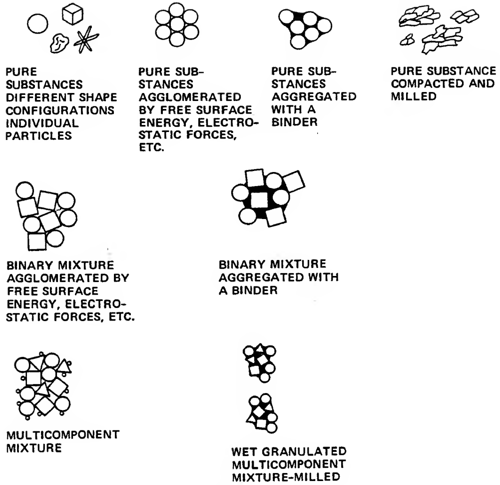

Figure 21 Several different types of particles encountered in tablet granulation dry blending.

may be found in the reference text: *Handbook of Pharmaceutical Excipients* [33].

Large (sieve size range >60 mesh) dry particles have a tendency to flow better than the smaller dry particles, because they have greater mass. Smaller particles (<100 mesh) may create mixing problems because surface areas are very great, and may give rise to strong electrostatic forces as a result of processing and/or inter-particle friction from movement. These forces may prevent the desired distribution of these smaller particles throughout a mixture because of fine particle agglomeration.

As the particle size approaches 10 μm and below, weak polarizing electrical forces called van der Waals forces or cohesive forces also begin to affect the flow of the powder. Both van der Waals and electrostatic forces usually inhibit powder flow through particle agglomeration as mentioned above. However, in some instances improved flow results because

Table 5 Effect of Particle Size on Powder Flow

Particle size	Type of flow[a]	Reason
200 − 250 μm (10 − 60[b] mesh)	Flow is usually good if shape is not interfering	Mass of individual particles is relatively large
250 − 75 μm (60 mesh − 200 μm)	Flow properties may be a problem with many pure substances and mixtures	Mass of individual particles is small and increased surface area amplifies effects of surface forces
<100 − 75 μm	Flow becomes a problem with most substances	Cohesive forces or free surface energy forces are large as well as static electrical forces relative to particle size

[a] Assume particle shape is constant and does not interfere with flow.
[b] U.S. standard mesh size.

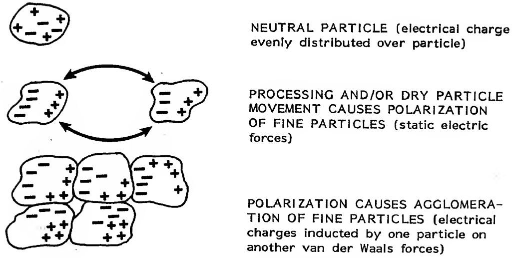

Figure 22 Effect of electrical forces on fine particles.

the agglomerated particles behave as a single large mass particle (Fig. 22). Flow may be better in this case, but the dynamics of distributing these small particles during mixing is very poor.

Increased surface exposure of fine particles to the atmosphere may present oxidation and/or moisture adsorption/absorption problems which should be avoided if possible. Fine powder particles also create potential dust conditions which may require operators to wear respirators for safe handling, and may also create potentially dangerous dust explosion hazards.

Particle size distribution of unit particles as suggested in the above discussion may also have an effect on the flow of a powder, i.e., too large a percentage of fine particles with cohesive forces, or free surface energy may inhibit flow. Although it has been stated that cohesive forces are strong in powders composed of particles 10 μm or less in size, each powder has a "critical size" where cohesive forces begin to affect the powder flow properties. An example of this is shown in Table 7.

The "angle of repose" (α) or the "angle of slip" is a relative measure of the friction between powder particles but also is a measure, for the most part, of the cohesiveness of fine particles. The angle of repose may be measured in several ways as shown in Figure 23. Methods 1 and 2 are both dynamic angle of repose measurements: the powder in Method 1 flows from a filled powder funnel onto a smooth surface where the angle is measured as illustrated, and in Method 2 the powder is moving in a rotating drum while the angle is measured as shown. Method 3 gives the static angle of repose, because the powder container is removed and the powder does not, or is not flowing before the measurement.

Since many factors enter into the angle of repose such as particle size, shape, moisture content, etc., there is some question as to its value in characterizing a powder. However, certain generalizations can be made regarding the angle of repose:

1. α is >60° for cohesive powders.
2. α is <25° for non-cohesive particles.
3. High (α) usually means poor powder flow and the particles are usually less than 75 to 100 μm in size.
4. Low (α) usually mean good powder flow and the particles are usually greater than 60 mesh or 250 μm in size.

The tangent of the angle repose ($\tan \alpha$) is termed the "coefficient of friction" of a powder and is preferred by some in referring to the flow properties of a powder. For example, a powder with an angle of repose of 65° will have a coefficient of friction of

$$\tan 65° = 2.14$$

Whereas, a powder with an angle of repose of 35° will have a coefficient of friction of

$$\tan 35° = 0.700$$

Table 6 Flow Characteristics of Some Common Substances

Material	Working bulk density (gm/cm^3)	Type of powder	General comments on flow
Acrawax C	0.46	Very fluid powder	Dusty, slippery material
Ammonium chloride	0.75	Nonuniform powdered granules	May form hard lumps, as a result of hygroscopicity
Calcium carbonate	0.92	Fluid cohesive powder	Flow becomes very poor if powder is packed
	0.36	Cohesive powder	
di-Calcium phosphate	0.99	Uniform granules	Powder form is very dusty. Material is hygroscopic which reduces flowability
	1.31	Very fluid granules and powder	
Cellulose	0.09	Fibrous not free flowing and crystalline free flowing	Flow depends on size of fibers or crystals
Kaolin	0.48	Fluid powder	Dusty material which has poor flow when powder is packed excessively

Magnesium hydroxide	0.56	Fluid powder	Dusty material which is hygroscopic. Flowability is reduced considerably when powder is packed excessively
Sodium chloride	1.10	Uniform granule or fluid granules and powder	Material is very hygroscopic and cakes at relative humidity: 40–50% at room temperature
Sodium bicarbonate	0.96	Fluid cohesive powder	Very little dustiness. Material is hygroscopic which decreases flowability
	1.08	Uniform powdered granules	
Corn starch	0.56	Very fluid powder	Very dusty. Material is hygroscopic which decreases flowability
Talc	0.67	Fluid powder	The two density powders are slippery and very dusty. Material is hygroscopic which decreases flowability
	0.19	Fluid cohesive powder	
Titanium dioxide	0.56	Cohesive powder	Flow becomes extremely poor if packed
Zinc oxide	0.45	Fluid cohesive powder	Dusty, tends to lump. Flow becomes poor when packed. Some dustiness tends to lump. Flow becomes poorer when packed
	0.74	Cohesive powder	

Source: Carr, R. L., Jr., *Chem. Eng.*, Feb., *1*:69–72 (1965).

Table 1: Partial lists of the modes of drug delivery and the pharmaceutical and medicinal agents from Genarro, Alfonso R., "Remington's Pharmaceutical Sciences".

<u>**Modes of Drug Delivery**</u>	<u>**Pharmaceuticals and Medicinal Agents**</u>
Solutions, Emulsions, Suspensions and Extractives • Aromatic waters • Aqueous acids • Douches • Enemas • Gargles • Mouthwashes • Syrups • Mucilages • Collodions • Elixers • Glycerins • Inhalalations and Inhalants • Linaments • Lotions Parenteral • Intramuscular • Implants • Transdermal Intravenous Opthalmic • Packs • Intracameral Injections • Iontophoresis • Subconjunctival Injections • Retrobulbar Injections Medicated applications • Percutaneous Absorption • Ointments • Pastes • Powders • Creams • Plasters • Suppositories • Contraceptives Powders • Oral Powders • Dentifrices • Douche powders • Dusting Powders • Insufflations • Triturations	Topical Drugs • Demulcents • Emollients • Astringents • Antiperspirants • Irritants • Rubefacients • Vesicants • Sclerosing Agents • Caustics • Escharotics • Keratolytics Gastrointestinal Drugs • Gastric Antacids • Digestants • Antiemetics Blood, Fluids, Electrolytes, Hematologic Drugs • Plasma Expanders • Antibodies • Isoagglutinins • Clotting agents • Anticoagulants • Hematopoietics Cardiovascular Drugs • Antihypertensive and Hypotensive Drugs • Vasopressor Drugs • Cardiac Glycosides • Antidysrhythmic Drugs Respiratory Drugs • Respiratory Stimulants • Antitussives • Bronchodilators • Therapeutic Gasses Sympathomimetic Drugs Cholinomimetic Drugs • Anticholinesterases • Cholinesterase reactivators Adrenergic Adrenergic Neuron Blocking Drugs Antimuscarinic Antispasmodic Drugs Skeletal Muscle Relaxants

Oral solid dosage forms • Tablets • Capsules • Pills • Trouches • Cachets • Pellets • Coatings Sustained release • Dissolution • Osmotic Systems • Ion Exchange Aerosols	• Neuromuscular Blocking Drugs • Muscle Relaxants • Antiparkinson Drugs Diuretic Drugs • Renal Tubular Inhibiting Diuretics Uterine Drugs Antimigraine Drugs Hormones • Adrenal Hormones • Pancreatic Hormones • Hypoglycemic/Hyperglycemic Drugs • Thyroid Hormones • Sex Hormones • Testicular Hormones Vitamins Nutrients Enzymes General Anesthetics Local Anesthetics Sedatives and Hypnotics • Benzodiapines • Barbiturates Antiepileptics Psychopharmacologic Agents • Antipsychotic Agents • Antianxiety Agents • Antidepressants • Analgesics and Antipyretics • Opiate Analgesics Histamine and Antihistamines • Inhibitors of Histamine Release Central Nervous System Stimulants • Xanthine Derivatives • Analeptics • Psychostimulants Antineoplastic Drugs Immunosuppressive Drugs Antimicrobial Drugs • Antiseptics • Disinfectants • Spermicides • Antibiotics • Antimalarials • Antifungal Drugs • Antiviral Drugs Parasiticides Pesticides Diagnostic Drugs

X. RELATED PROCEEDINGS APPENDIX

None.